Appendix A

ROBUST SUMMARY FOR 2-METHYL-3-BUTENENITRILE

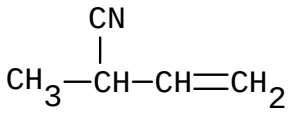
Existing published and unpublished data were collected and scientifically evaluated to determine the best possible study or studies to be summarized for each required endpoint. In the spirit of this voluntary program, other data of equal or lesser quality are not summarized, but are listed as related references at the end of each appropriate section, with a statement to reflect the reason why these studies were not summarized.

1.0 Substance Information

CAS Number: 16529-56-9

Chemical Name: 3-Butenenitrile, 2-methyl

Structural Formula:



Other Names: 3-Cyanobut-1-ene
2-Methyl allylcyanide
2M3BN
3-Cyanobutene-1

Exposure Limits: No Data.

2.0 Physical/Chemical Properties

2.1 Melting Point:

Value:	-59.98°C
Decomposition:	No Data
Sublimation:	No Data
Pressure:	760 mm Hg
Method:	Modeled. MPBPWIN, v.1.4, module of EPIWIN 3.05 (Syracuse Research Corporation). MPBPWIN estimates melting point by two different methods. The first is an adaptation of the Joback group contribution method for melting point (Joback, 1982; Reid et al., 1987) and the second is a simple Gold and Ogle method suggested by Lyman, 1985.
GLP:	Not Applicable
Reference:	Joback, K. G. (1982). A Unified Approach to Physical Property Estimation Using Multivariate Statistical Techniques. Stevens Institute of Technology, submitted to the Dept. of Chem. Eng. For M.S. Degree at the Massachusetts Institute of Technology in June 1984 (see also: Reid et al., 1987).

Reid, R. C. et al. (1987). The Properties of Gases and Liquids, 4th edition, Chapter 2, Mc-Graw-Hill, Inc., NY.

Lyman, W. J. (1985). In: Environmental Exposure From Chemicals, Volume I, Chapter 2, Neely, W. B. and G. E. Blau (eds.), CRC Press, Inc., Boca Raton, FL.

Reliability: Estimated value based on accepted model.

SUPPORTING DATA: 1-PENTENENITRILE

Value:	-96°C
Decomposition:	No Data
Sublimation:	No Data
Pressure:	No Data
Method:	No Data
GLP:	No Data
Reference:	Lievens (1924). Bull. Soc. Chim. Belg., 22:127 (Beilstein Database, accessed June 17, 2003).
	Timmermans (1927). Bull. Soc. Chim. Belg., 36:507 (Beilstein Database, accessed June 17, 2003).
	Timmermans and Delcourt (1934). J. Chim. Phys. Phys. Chim. Biol., 31:110 (Beilstein Database, accessed June 17, 2003).
	Joutkovsky (1934). Bull. Soc. Chim. Belg., 43:401 (Beilstein Database, accessed June 17, 2003).
	Witschonke (1954). Anal. Chem., 26:562 (Beilstein Database, accessed June 17, 2003
Reliability:	Not assignable because limited study information was available.

SUPPORTING DATA: 9-OCTADECENENITRILE

Value:	-1°C
Decomposition:	No Data
Sublimation:	No Data
Pressure:	No Data
Method:	No Data
GLP:	Unknown
Reference:	Weast, R.C. (ed.) (1979). Handbook of Chemistry and Physics, 60th ed., p. C-404, CRC Press Inc., Boca Raton, Florida.

Reliability:	Not assignable because limited study information was available.

Additional References for Melting Point: None Found.

2.2 Boiling Point

Value:	121-145°C
Decomposition:	Decomposes with heat
Pressure:	760 mm Hg
Method:	No Data
GLP:	Unknown
Reference:	DuPont Co. (2000). Material Safety Data Sheet No. DU000187 (June 24).
Reliability:	Not assignable because limited study information was available.

Additional References for Boiling Point: None Found.

2.3 Density

Value:	0.8
Temperature:	25°C
Method:	No Data
GLP:	Unknown
Results:	No additional data.
Reference:	DuPont Co. (2000). Material Safety Data Sheet No. DU000187 (June 24).
Reliability:	Not assignable because limited study information was available.

Additional References for Density: None Found.

2.4 Vapor Pressure

Value:	11.1 mm Hg
Temperature:	25°C
Decomposition:	No Data
Method:	Estimated using the mean of Antoine and Grain methods.
GLP:	Not Applicable
Reference:	SRC MPBPWIN v1.40 in EPIWIN v3.05.
	Syracuse Research Corporation (MPBPWIN) program estimates the boiling point (at 760 mm Hg), melting point, and vapor pressure of organic compounds. The vapor pressure is estimated using the mean of the Antoine and

Grain methods. A description of the methodology is detailed in:

Antoine Method: Lyman, W. J. et al. (1990). Handbook of Chemical Property Estimation Methods, Chapter 14, American Chemical Society, Washington, DC.

Modified Grain Method: Lyman, W. J. (1985). In: Environmental Exposure From Chemicals, Volume I, Chapter 2, Neely, W. B. and G. E. Blau (eds.), CRC Press, Inc., Boca Raton, FL.

Reliability: Estimated value based on accepted model.

Additional Reference for Vapor Pressure:

DuPont Co. (2000). Material Safety Data Sheet No. DU000187 (June 24).

2.5 Partition Coefficient (log Kow)

Value:	1.12
Temperature:	Not Applicable
Method:	Modeled. The KOWWIN computer program, version 1.66 from Syracuse Research Corporation, calculates the Log octanol/water partition coefficient (log Kow) of organic chemicals using an atom/fragment contribution method.
GLP:	Not Applicable
Reference:	The methodology is described in the following journal article: Meylan, W. M. and P. H. Howard (1995). J. Pharm. Sci., 84:83-92.
Reliability:	Estimated value based on accepted model.

Additional References for Partition Coefficient (log Kow): None Found.

2.6 Water Solubility

Value:	6917 mg/L
Temperature:	22.5°C
pH/pKa:	No Data
Method:	Water solubility of the test substance was estimated by determining the total organic carbon in water to which an amount of test substance in excess of the water solubility had been added. Samples were tested for their solubility in water after 48 and 96 hours of continuous mixing. Approximately 200 mg of the test substance, prepared in quadruplicate

	replicates, was added to 20 mL of deionized water in a glass test vessel with a Teflon®-coated screw cap. Two of the replicates were mixed end-over-end for 48 hours and then analyzed. The other 2 replicates were mixed end-over-end for 96 hours before analysis. Prior to analysis, the test vessels were allowed to stand for 1 hour. The upper 5 mL layer of solution was removed by pipette and discarded to eliminate test substance at the top of the test vessel. Samples were analyzed for total carbon content via an analyzer with an autosampler attachment. Water solubility was determined by assuming that the test material contained 100% test substances.
GLP:	No
Reference:	DuPont Co. (2002). Unpublished Data, Report No. EMSER 007-02, "Estimated Water Solubility of 2-Methyl-3-Butenenitrile" (January 24).
Reliability:	Medium because a suboptimal study design was used

Value:	7850 mg/L
Temperature:	25°C
pH/pKa:	No Data
Method:	Modeled
GLP:	Not Applicable
Reference:	WsKow v1.4 in EPIWIN v3.05 (SRC Database).
	WsKow estimates the water solubility (Wsol) of an organic compound using the compound's log octanol-water partition coefficient (log Kow). The following journal article describes the estimation methodology:
	Meylan, W. M. et al. (1996). Environ. Toxicol. Chem., 15:100-106.
Reliability:	Estimated value based on accepted model.

Additional Reference for Water Solubility:

DuPont Co. (2000). Material Safety Data Sheet No. DU000187 (June 24).

2.7 Flash Point

Value:	15°C
Method:	Closed cup
GLP:	Unknown
Reference:	DuPont Co. (2000). Material Safety Data Sheet No. DU000187 (June 24).
Reliability:	Not assignable because limited study information was

available.

Additional References for Flash Point: None Found.

2.8 Flammability

Results:	Flammable liquid; vapor forms explosive mixture with air.
Method:	No Data
GLP:	Unknown
Reference:	DuPont Co. (2000). Material Safety Data Sheet No. DU000187 (June 24).
Reliability:	Not assignable because limited study information was available.

Additional References for Flammability: None Found.

3.0 Environmental Fate

3.1 Photodegradation

Concentration:	Not Applicable
Temperature:	Not Applicable
Direct Photolysis:	Using the absorption spectrum of acetonitrile as an analog example, the nitrile group does not absorp significantly above 200 nm: absorbance at 200 nm = 0.04 absorbance at 210 nm = 0.03 absorbance at 220 nm = 0.01 absorbance at 254 nm = 0.005. Harris (1990) also reported that ethylene, an analog for C=C in the unsaturated mononitriles, has no significant absorption above 290 nm. Therefore, the mononitrile category is expected to lack significant absorptivity above 290 nm and will not be subject to direct photolysis.
Indirect Photolysis:	In the vapor phase, 2 methyl-3-butenenitrile, is estimated to have an atmospheric half-life of 0.6 days due to hydroxyl radical oxidation and a half-life of 0.96 days due to reactions with ozone. The two reactions result in an estimated atmospheric half-life of 0.37 days for vapor phase material.
Breakdown Products:	Not Applicable
Method:	The AOP Program, version 1.90 from Syracuse Research Corporation, estimates the Atmospheric Oxidation Potential. The AOP program estimates the rate constant for the atmospheric, gas-phase reaction between photochemically

produced hydroxyl radicals and organic chemicals. The methodology used by the Atmospheric Oxidation Program is based upon the structure-activity relationship (SAR) methods developed by Dr. Roger Atkinson and co-workers (Atkinson et al., 1987; 1995; 1996; 1984).

The rate constant for the reaction of 2-methyl-3-butenenitrile vapor with photochemically generated hydroxyl radicals in the atmosphere is estimated to be $2.6x10^{-11}$ cm^3/molecule-sec at 25°C (SRC AOPWin v1.90). This value corresponds to a half-life of 0.6 days, assuming a 24 hour day and an ambient hydroxyl radical concentration of $0.5x10^6$ molecules/cm^3.

GLP: Not Applicable

Reference: Atkinson, R. et al. (1987). Intern. J. Chem. Kinet., 19 :799-828.

Atkinson, R. et al. (1995). Atmos. Environ., 29:1685-1695.

Atkinson, R. et al. (1996). Environ. Sci. Technol., 30:329-334.

Atkinson, R. et al. (1984). Chem. Rev., 84:437-470.

Harris, J. C. (1990). Rate of Aqueous Photolysis, Chapter 8, In: Lyman, W. J. et al. (eds.). Handbook of Chemical Property Estimation Methods, American Chemical Society, Washington, DC.

The following journal article describes the AOP Program:

Meylan, W. M. and P. H. Howard (1993). Chemosphere, 26:2293-2299.

Reliability: Estimated value based on accepted model.

Additional References for Photodegradation: None Found.

3.2 Stability in Water

Concentration: Not Applicable

Half-life: The Henry's Law constant for 2-methyl-3-butenenitrile is estimated to be $5.33x10^{-5}$ atm-m^3/mole (SRC HENRYWIN v3.10 in EPIWIN v3.05) from its estimated vapor pressure of 11.1 mm Hg (SRC MPBPWIN v1.40 in EPIWIN v3.05, mean of Antoine & Grain methods) and water solubility of 7850 mg/L (WsKow v1.40 in EPIWIN v 3.05). The

	estimated volatilization half-life from a model river (1 m deep, flowing 1 m/sec, wind velocity of 5 m/sec) is approximately 10.8 hours. The estimated volatilization half-life from a model lake (1 m deep, flowing 0.05 m/sec, wind velocity of 0.5 m/sec) is approximately 8.1 days (EPIWIN v3.05).
% Hydrolyzed:	Not Applicable
Method:	Modeled. The WVOL program estimates the volatilization half-lives from a model river and lake using the methodology from Lyman et al., 1990 (adsorption to suspended solids and sediments is ignored). WsKow estimates the water solubility (Wsol) of an organic compound using the compound's log octanol/water partition coefficient (log Kow).
GLP:	Not Applicable
Reference:	Lyman, W. J. et al. (1990). The Handbook of Chemical Property Estimation Methods, American Chemical Society, Washington, DC. The following journal article describes the estimation methodology: Meylan, W. M. et al. (1996). Environ. Toxicol. Chem., 15:100-106.
Reliability:	Estimated value based on accepted model.

Additional References for Stability in Water: None Found.

3.3 Transport (Fugacity)

Media:	Air, Water, Soil, and Sediments	
Distributions:	Air:	1.9%
	Water:	44.1 %
	Soil:	53.9%
	Sediments:	0.09%
Half-life:	Air:	8.8 hours
	Water:	360 hours
	Soil:	720 hours
	Sediment:	3240 hours
Adsorption Coefficient:	Not Applicable	
Desorption:	Not Applicable	
Volatility:	Not Applicable	
Method:	Calculated according to Mackay, Level III, Syracuse Research Corporation EPIWIN version 3.05. Emissions	

(1000 kg/hr) to air, water, and soil compartments using standard EPA model defaults with BIOWIN half-life factors of water, 1; soil, 2; and sediments, 9.

Data Used:
Molecular Weight: 81.12
Chemical Name: 2-methyl-3-butenenitrile
Henry's Law Constant: 5.33 x 10^{-5} atm-m^3/mole (HenryWin Program)
Vapor Pressure: 11.1 mm Hg (MPBPWIN v1.40)
Log Kow: 1.12 (KowWin Program)
Soil Koc: 5.4 (Log Kow estimate)

GLP: Not Applicable

Reference: Syracuse Research Corporation EPIWIN v3.05 contains a Level III fugacity model. The methodology and programming approach were developed by Dr. Donald MacKay and coworkers and are detailed in:

Mackay, D. (1991). Multimedia Environmental Models: The Fugacity Approach, pp. 67-183, Lewis Publishers, CRC Press.

Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1618-1626.

Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1627-1637.

Reliability: Estimated value based on accepted model.

Additional References for Transport (Fugacity): None Found.

3.4 Biodegradation:

Value: 8% after 21 days (Not Readily Biodegradable)

Breakdown Products: No Data

Method: The procedures used in the test were based on the recommendations of the following guideline:

OECD Guideline 301B.

The biodegradability of 2-methyl-3-butenenitrile was tested using the Modified Sturm test. Biodegradability was measured as CO_2 evolution. A test substance is considered "Readily Biodegradable" if it demonstrates a "pass level" of 60% biodegradability within a 10-day window after

	exceeding the 10% level of biodegradability. A test substance is considered "Ultimately Biodegradable" if it demonstrates a "pass level" of 60% biodegradability, but not within a 10-day window after exceeding the 10% level of biodegradability. 2-Methyl-3-butenenitrile reached a peak of 8% biodegradability at day 21, and therefore is regarded as not "Readily Biodegradable." 2-Methyl-3-butenenitrile was not inhibitory to microorganisms in the inoculum.
GLP:	No
Reference:	DuPont Co. (2001). Unpublished Data, Report No. EMSE-072-01, "Biodegradability of 2-Methyl-3-Butenenitrile Using the Modified Sturm Test (OECD 301B)" (December 17).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Biodegradation: None Found.

3.5 Bioconcentration

Value:	BCF = 1.45. This BCF value suggests that bioconcentration potential in aquatic organisms is low.
Method:	The bioconcentration factor is calculated by Syracuse Research Corporation's BCFWIN Computer Program, version 2.14, which utilizes a linear regression based on the log Kow for the compound.
GLP:	Not Applicable
Reference:	The estimation methodology used by BCFWIN is described in the following document prepared for the U.S. Environmental Protection Agency (OPPT): "Improved Method for Estimating Bioconcentration Factor (BCF) from Octanol-Water Partition Coefficient," SRC TR-97-006 (2^{nd} Update), July 22, 1997; prepared for Robert S. Boethling, EPA-OPPT, Washington, DC, Contract No. 68-D5-0012; prepared by William M. Meylan, Philip H. Howard, Dallas Aronson, Heather Printup, and Sybil Gouchie, Syracuse Research Corp., Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212.
Reliability:	Estimated value based on accepted model.

Additional References for Bioconcentration: None Found.

4.0 Ecotoxicity

4.1 Acute Toxicity to Fish

Type:	**96-hour LC_{50}**
Species:	*Pimephales promelas* (fathead minnow)
Value:	>100 mg/L
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used that was consistent with OECD Guideline 203, with the following exceptions: 10x dose spacing, 4 test concentrations, and nominal test concentrations were reported. The acute toxicity to fathead minnows was determined in an unaerated, 96-hour, static test. The nominal concentrations of 2-methyl-3-butenenitrile used were 0, 0.10, 1, 10, and 100 mg/L at a mean temperature of 21.6°C. One test chamber was used per test concentration with 10 test organisms in each chamber. Analysis of the test and control solution samples for dissolved oxygen and pH were made at test initiation (0 hours) and test completion (96 hours).
GLP:	No
Test Substance:	2-Methyl-3-butenenitrile, purity 87%
Results:	Based on visual observations, the water control and the 0.1, 1, 10, and 100 mg/L test concentrations were clear and colorless at test start. All water quality parameters were within acceptable limits during the exposure. At test initiation (0 hours), dissolved oxygen was 8.7 mg/L and pH ranged from 7.5-7.7. At test completion (96 hours) dissolved oxygen and pH ranged from 6.1-7.1 and 7.4-7.6, respectively. Exposure of fathead minnows to nominal concentrations of 0, 0.1, 1, 10, and 100 mg/L 2-methyl-3-butenenitrile resulted in 0% mortality at any concentration at the end of 96 hours. The test substance exhibited low concern for aquatic hazard in the unaerated, 96-hour, static, acute test using the fathead minnow.
Reference:	DuPont Co. (2001). Unpublished Data, Haskell Laboratory Report No. DuPont-8177, "Static, Acute, 96-Hour Screening Test to *Pimephales promelas*" (November 19).
Reliability:	Medium because a suboptimal study design was used (nominal test concentrations).

Type:	**96-hour LC_{50}**
Species:	Fathead minnow
Value:	473 µg/L; log Kow = 1.12
Method:	Estimated
GLP:	Not Applicable
Test Substance:	2-Methyl-3-butenenitrile
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

SUPPORTING DATA: 3-BUTENENITRILE

Type:	**96-hour LC_{50}**
Species:	*Pimephales promelas* (fathead minnow)
Value:	182 mg/L (confidence limits, 171-195 mg/L)
Method:	Gas-liquid chromatography (GLC) was used to analyze toxicants in water samples from the fish exposure tanks. All test chambers were sampled at approximately mid-depth at 0, 24, 48, 72, and 96 hours in all exposure chambers. All samples were analyzed immediately or adequately preserved for later analysis. Five water quality parameters (temperature, dissolved oxygen, total hardness, total alkalinity, and pH) were routinely measured for each test. Temperature measurements were made in each exposure chamber daily. Dissolved oxygen was determined in the high, medium, low, and control exposure chambers at least 3 times (0, 24, and 96 hours) during a test if surviving fish existed in that chamber. Total hardness and total alkalinity were determined at least once for each test. pH was measured at least once during each test in high, medium, low, and control exposure chambers. Fathead minnows (approximately 33 days old) were exposed to nominal concentrations of 0, 73.3, 113, 173, 267, and 410 mg/L. Fish were not fed during chemical exposure. During the exposure, fish were routinely observed for behavioral responses (effects) and deaths. Death was defined as the cessation of opercular movements and the

inability to respond when prodded. Dead fish were removed and recorded at 3, 6, 12, 24, 48, 72, and 96 hours from initial exposure. At the termination of tests, control fish were weighed (wet) to the nearest mg after blotting excess water from them with a paper towel and measured (standard length) to the nearest mm.

Exposure of fish was done in flow-through exposures, with a modified continuously proportioning diluter without duplicate exposures. The modification was the elimination of flow booster and self-siphoning flow splitting cells. Diluters were calibrated. The test substance was proportionally diluted with Lake Superior water from stock solutions before delivery to fish exposure chambers.

GLP: No

Test Substance: 3-Butenenitrile, purity 98.6%

Results: Average measured test concentrations were 73.8, 105, 166, 240, and 350 mg/L for the 0, 73.3, 113, 173, 267, and 410 mg/L nominal concentrations. Measured water quality parameters included temperature of 25°C, dissolved oxygen of 5.9 mg/L, hardness of 46.0 mg/L $CaCO_3$, alkalinity of 42.0 mg/L $CaCO_3$, and pH of 7.70.

Mortality at 96 hours was 0/20, 0/20, 0/20, 3/20, 20/20, and 20/20 at 0, 73.8, 105, 166, 240, and 350 mg/L. Affected fish had abdominal swelling and lost equilibrium prior to death.

Reference: Brooke, L. T. et al. (eds.) (1984). Acute Toxicities of Organic Chemicals to Fathead Minnows (*Pimephales promelas*), Center for Lake Superior Environmental Studies, University of Wisconsin-Superior, Superior, Wisconsin.

Reliability: High because a scientifically defensible or guideline method was used.

Type: **96-hour LC_{50}**

Species: Fathead minnow

Value: 447 μg/L; log Kow = 0.7

Method: Estimated

GLP: Not Applicable

Test Substance: 3-Butenenitrile

Results: No additional data.

Reference: Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center,

	Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Fish: None Found.

4.2 Acute Toxicity to Invertebrates

Type:	**48-hour EC_{50}**
Species:	*Daphnia magna*
Value:	>100 mg/L
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used that was consistent with OECD Guideline 202, with the following exceptions: 10x dose spacing, 4 test concentrations, nominal test concentrations were reported, and 1 replicate per test concentration was performed. The acute toxicity of 2-methyl-3-butenenitrile to the water flea, *Daphnia magna* (less than 24-hours old) was determined in an unaerated, 48-hour, static test. The study was conducted at concentrations of 0, 0.1, 1.0, 10, and 100 mg/L at a mean temperature of 20.4°C (range of 20.1-20.7°C). One test chamber was used per test concentration with 10 test organisms in each chamber.
GLP:	No
Test Substance:	2-Methyl-3-butenenitrile, purity 87%
Results:	Based on visual observations, the water control and the 0.1, 1, 10, and 100 mg/L test concentrations were clear and colorless at test start. All water quality parameters were within acceptable limits during the exposure. Dissolved oxygen was 8.6-8.7 and 8.4-8.5 mg/L at test initiation (0 hours) and test completion (48 hours), respectively. pH was 7.4-7.9 and 7.7-7.9 at test initiation (0 hours) and test completion (48 hours), respectively. Immobility was 0% in all test concentrations at the end of 48 hours. No immobility or sublethal effects were observed in the water control test organisms. The highest nominal concentration causing no immobility at test end was 100 mg/L. The test substance exhibited low concern for aquatic hazard in an unaerated, 48-hour, static, acute test using *Daphnia magna* (less than 24 hours old).
Reference:	DuPont Co. (2001). Unpublished Data, Haskell Laboratory Report No. DuPont-8178, "Static, Acute, 48-Hour Screening Test to *Daphnia magna*" (October 26).

Reliability:	Medium because a suboptimal study design was used (nominal test concentrations).

Additional References for Acute Toxicity to Invertebrates: None Found.

4.3 Acute Toxicity to Aquatic Plants: No Data.

5.0 Mammalian Toxicity

5.1 Acute Toxicity

Type:	**Oral ALD**
Species/Strain:	Male rats/ChR-CD
Value:	1000 mg/kg
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. 2-Methyl-3-butenenitrile, as a solution in peanut oil, was administered in single doses via intragastric intubation to young adult male rats (1/dose level) at 200, 300, 450, 670, 1000, 1500, 2250, or 5000 mg/kg. Survivors were sacrificed 14 days later without pathological examination. Clinical signs of toxicity and body weights were recorded.
GLP:	No
Test Substance:	2-Methyl-3-butenenitrile, purity approximately 100%
Results:	Mortality occurred at doses ≥ 1000 mg/kg within 5 days. Toxic signs observed at lethal doses included salivation, increased muscle tone, polyuria, irregular respiration, weight loss, hyperemic extremities, and unresponsiveness. The sublethal dose of 670 mg/kg caused weight loss for 3 days, salivation, increased muscle tone, rapid respiration, and inactivity. Throughout most of the recovery period, this rat was unkempt, emaciated, and exhibited nervous behavior. At 450 and 300 mg/kg, the rats showed toxic signs during the 1st recovery week, which included weight loss, polyuria, nervous behavior, hyperemia, and ruffled fur. Other than an initial weight loss, there were no effects observed in the rat dosed at 200 mg/kg.
Reference:	DuPont Co. (1967). Unpublished Data, Haskell Laboratory Report No. 199-67, "Acute Oral Test" (November 9) (also cited in TSCA fiche OTS0571510).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Oral Toxicity: None Found.

Type:	**Inhalation LC_{50}**
Species/Strain:	Male rats/ChR-CD
Exposure Time:	4 hours
Value:	3000 ppm (95% confidence limits, 2760-3261 ppm)
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Male rats (6/exposure level), weighing 250-289 g, were exposed to nominal concentrations of 815, 1060, 1540, 1812, 2416, 2870, 3200, or 3835, ppm 2-methyl-3-butenenitrile in a 16 L bell jar for 4 hours. The test substance was metered at a uniform rate into a heated stainless steel T-tube by a syringe drive and vaporized under prepurified nitrogen. The test substance vapors were mixed with oxygen and carried into the bell jar. Houseline air was used as diluent to give the desired atmospheric concentration. For analysis, gas samples were taken periodically from the chamber exit and analyzed by gas chromatography. Clinical signs were recorded during and post-exposure. Body weights were recorded. Gross and histopathologic examinations were performed on 2 rats each at 1, 2, 7, and 14 days post-exposure. Tissues examined included lungs, liver, spleen, kidney, testes, and thymus. The other survivors were sacrificed 14 days post-exposure.
GLP:	No
Test Substance:	2-Methyl-3-butenenitrile, purity approximately 100%
Results:	The analytical concentrations for the 815, 1060, 1540, 1812, 2416, 2870, 3200, and 3835 ppm exposure levels were not specified, not specified, not specified, 2480, 2600, 2945, 2875, and 3470 ppm, respectively. Mortality was 0/6, 0/6, 0/6, 0/6, 1/6, 1/6, 3/6, and 5/6 at 815, 1060, 1540, 1812, 2416, 2870, 3200, and 3835 ppm, respectively. Death occurred from 3.5 hours of exposure through the night following exposure. During exposure, clinical signs observed at lethal concentrations included irregular respiration, incoordination, lacrimation, salivation, inflamed eyes, red discharge from the eyes, hyperemia, pale ears, tremors, and unresponsiveness to sound. The same clinical signs, but less severe, were observed in animals at non-lethal doses. Post-exposure initial weight loss followed by normal weight gain was observed at lethal and non-lethal concentrations. Gross and histopathologic examinations revealed no anatomical evidence of primary injury.

Reference:	DuPont Co. (1970). Unpublished Data, Haskell Laboratory Report No. 301-70, "Acute Inhalation Toxicity" (July 15) (also cited in TSCA fiche OTS0555686). DuPont Co. (1968). Unpublished Data, Study Records (January 16).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Inhalation Toxicity: None Found.

Type:	**Dermal LD_{50}**
Species/Strain:	Male rabbits/New Zealand White
Exposure Time:	24 hours
Value:	482 mg/kg
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Four groups of 6 adult male rabbits were clipped free of hair over the back and trunk area and fitted with plastic collars. The test substance (250, 325, 400, or 700 mg/kg) was applied to the intact skin on the back of each rabbit under a gauze pad. The trunk of each rabbit was then wrapped with a layer of plastic wrap, stretch gauze bandage, and elastic adhesive tape. After a 24-hour exposure period, the wrappings were removed, and the rabbits were wiped with a dry towel and returned to their cages. The rabbits were observed and/or weighed daily (except weekends) over a 14-day recovery period and then sacrificed. The LD_{50} value was calculated using the method of D. J. Finney.
GLP:	No
Test Substance:	2-Methyl-3-butenenitrile, purity 74%
Results:	Mortality was 0/6, 2/6, 3/6, and 4/6 at 250, 325, 400, and 700 mg/kg, respectively. All deaths occurred within 1 day after dosing. Weight loss was observed at all dose levels tested. Clinical signs of toxicity observed on the day of dosing or the day following dosing included prostration (one rabbit at 700 mg/kg) and weakness (one rabbit each at 400 and 700 mg/kg). In addition one rabbit at 250 mg/kg was observed not to be eating on day 5 following dosing.
Reference:	DuPont Co. (1983). Unpublished Data, Haskell Laboratory Report No. 40-83, "Acute Skin Absorption LD_{50} Test on Rabbits" (March 1) (also cited in TSCA fiche OTS0570961).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Dermal Toxicity: None Found.

Type:	**Dermal Irritation**
Species/Strain:	Male guinea pigs/Albino
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. In a test for primary irritation, applications of 1 drop of the undiluted sample (100%) or of a solution in 1:1 acetone-dioxane containing 13% guinea pig fat (f.a.d.) were applied to the intact shaved skin of 10 male albino guinea pigs. The final concentrations were 100%, 50%, and 25%. Reactions were observed after 1 and 2 days.
GLP:	No
Test Substance:	2-Methyl-3-butenenitrile, purity approximately 100%
Results:	No skin reaction was observed 1 or 2 days after treatment with 100%, 50% (observed only 1 day after treatment), or 25% 2-methyl-3-butenenitrile.
Reference:	DuPont Co. (1969). Unpublished Data, Haskell Laboratory Report No. 230-69, "Primary Skin Irritation and Sensitization Tests" (August 13).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Dermal Irritation: None Found.

Type:	**Dermal Sensitization**
Species/Strain:	Male guinea pig/Albino
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. In the test for sensitization potential, an exposure series was given during a 3-week interval. Five guinea pigs received 9 applications of 100% 2-methyl-3-butenenitrile, and 5 others received 4 intradermal injections (each 0.1 mL of a 1% solution, based on corrected specific gravity value, in dimethyl phthalate). A 2-week rest period was followed by a challenge test consisting of applications of 100% test substance and 50% solution (based on corrected specific gravity value) in 1:1 acetone-dioxane containing 13% guinea pig fat (f.a.d.) to both intact and abraded skin. Sensitization reactions were observed at 1 and 2 days.
GLP:	No

Test Substance:	2-Methyl-3-butenenitrile, purity approximately 100%
Results:	Sensitization reactions at the challenge phase included 1 guinea pig with mild erythema at 100% in intact skin at the 1-day observation. All other readings for intact and abraded skin were negative. 2-Methyl-3-butenenitrile was not a skin sensitizer when tested in albino guinea pigs.
Reference:	DuPont Co. (1969). Unpublished Data, Haskell Laboratory Report No. 230-69 "Primary Skin Irritation and Sensitization Tests" (August 13).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Dermal Sensitization: None Found.

Type:	**Eye Irritation**
Species/Strain:	Rabbits/Albino
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Undiluted 2-methyl-3-butenenitrile (0.1 mL) was instilled into the right conjunctival sac of each of 2 albino rabbits. Twenty seconds after contact, 1 exposed eye was washed with tap water for 1 minute. The exposed eye of the other rabbit was not washed. Observations were made with a hand slit lamp at 1 and 4 hours, and at 1, 2, 3, and 7 days. Fluorescein stain and a biomicroscope were used at examinations after the day of treatment.
GLP:	No
Test Substance:	2-Methyl-3-butenenitrile, purity approximately 100%
Results:	2-Methyl-3-butenenitrile produced temporary, very mild corneal injury and conjunctival irritation in the unwashed rabbit eye. Another eye similarly dosed and promptly washed showed only temporary, mild conjunctival irritation.
Reference:	DuPont Co. (1969). Unpublished Data, Haskell Laboratory Report No. 230-69, "Eye Irritation Test" (August 13).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Eye Irritation: None Found.

5.2 Repeated Dose Toxicity

Type:	**2-Week Inhalation Study**
Species/Strain:	Rats/ChR-CD
Sex/Number:	Male/6
Exposure Period:	2 weeks (total of 10 exposures)
Frequency of Treatment:	4 hours/day
Exposure Level:	560 ppm
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Six male rats, weighing 250-289 g, were exposed to 2-methyl-3-butenenitrile in a 16 L bell jar 4 hours/day for 2 weeks. The test substance was metered at a uniform rate into a heated stainless steel T-tube by a syringe drive and vaporized under prepurified nitrogen. The test substance vapors were mixed with oxygen and carried into the bell jar. Houseline air was used as diluent to give the desired atmospheric concentration. For analysis, gas samples were taken periodically from the chamber exit and analyzed by gas chromatography. Clinical signs and body weights were recorded during and post-exposure. Gross and histopathologic examinations were performed, and included lungs, liver, spleen, kidney, testes, and thymus.
GLP:	No
Test Substance:	2-Methyl-3-butenenitrile, purity approximately 100%
Results:	At 560 ppm, no mortality was observed. Clinical signs during exposure included irregular respiration, hypersensitivity, red discharge around the eyes, salivation, pale ears, piloerection (during the 5th, 6th, and 8th exposures), and no weight gain during the exposure period. Post-exposure, animals had normal weight gain, and no clinical signs were observed. Gross and histopathologic examination showed no evidence of primary injury by the test substance.
Reference:	DuPont Co. (1970). Unpublished Data, Haskell Laboratory Report No. 301-70, "Subacute Inhalation Toxicity" (July 15) (also cited in TSCA fiche OTS0555686).
Reliability:	Medium because a suboptimal study design was used.

Additional References for Repeated Dose Toxicity: None Found.

5.3 Developmental Toxicity: No Data.

5.4 Reproductive Toxicity: No Data.

5.5 Genetic Toxicity

Type:	***In vitro* Bacterial Reverse Mutation Test**
Tester Strain:	*Salmonella typhimurium* strains TA97, TA98, TA100, TA104, TA1535, and TA1537
Exogenous Metabolic Activation:	With and without 10 and 30% Aroclor®-induced rat and hamster liver S-9
Exposure Concentrations:	Initial Trial: 0, 10, 33, 100, 333, 1000, 3333, and 6666 or 6667 µg/plate Subsequent Trials: 0, 33, 100, 333, 1000, 3333, and 6666 or 6667 µg/plate Comment: Not all exposure concentrations were tested with all tester strains under all test conditions.
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. The preincubation method originally described by Haworth et al., 1983 was used with some modifications. The test substance, overnight culture of *Salmonella*, and S-9 mix or buffer were incubated at 37°C, without shaking for 20 minutes. Test substances known or suspected to be volatile were incubated in capped tubes. The top agar was added and the contents of the tubes were mixed and poured onto the surface of petri dishes containing medium. Histidine-independent (his+) colonies arising on these plates were counted following 2 days incubation at 37°C. Plates were machine counted (New Brunswick, Artek). At the discretion of the investigator, plates with low numbers of colonies, containing precipitated test substance, or having excessively-reduced contrast because of chemical color, were counted by hand. The initial test of a test substance was without activation and with 10% S-9. If a positive result was obtained, the positive trial(s) was repeated. If the trials were negative the test substance was retested without S-9 and with 30% S-9. If all trials were negative, no further testing was performed. A test substance was designated nonmutagenic only after it had been tested in strains TA97, TA98, TA100, TA1535,

and TA1537, without exogenous activation, and with 10% and 30% rat and hamster S-9.

2-Methyl-3-butenenitrile was run initially in a toxicity assay using TA100 or the system developed by Waleh et al., 1982. Toxic concentrations were defined as those that produced a decrease in the number of his+ colonies, or a clearing in the density of the background lawn, or both.

The test substance was initially tested in the preincubation test at half-log dose intervals up to a dose that elicited toxicity, or to a dose immediately below one that was toxic in the preliminary toxicity procedure. Subsequent trials occasionally used narrower dose increments, and may not have included doses in the toxic range. At least 5 doses of the test substance were tested in triplicate, and repeat experiments were performed at least 1 week following the initial trial.

Concurrent solvent (dimethyl sulfoxide) and positive controls were run with each trial. The positive controls in the absence of exogenous metabolic activation were sodium azide (TA1535 and TA100), 9-aminoacridine (TA97 and TA1537), and 4-nitro-o-phenylenediamine (TA98). The positive control for exogenous metabolic activation with all strains was 2-aminoanthracene.

The test substance was considered mutagenic or weakly mutagenic if it produced a reproducible, dose-related response over the solvent control, under a single metabolic activation condition, in replicate trials. The test substance was considered questionable if the results of individual trials were not reproducible, if increases in his+ revertants did not meet the criteria for a weakly positive response, or if only single doses produced increases in his+ revertants in repeat trials. The test substance was judged nonmutagenic if it did not meet the criteria for a mutagenic or questionable response.

GLP: Unknown
Test Substance: 2-Methyl-3-butenenitrile, purity 82%
Results: Equivocal
Remarks: 2-Methyl-3-butenenitrile was cytotoxic as indicated by a slight clearing of the background lawn and a reduction of revertants starting at concentrations of 6666 μg/plate in the presence of metabolic activation. In the non-activated condition, indication of toxicity was observed with some

strains starting at 3333 µg/plate. Weakly mutagenic or equivocal results were produced without exogenous activation in *Salmonella typhimurium* strain TA97. 2-Methyl-3-butenenitrile was nonmutagenic with or without exogenous activation in *Salmonella typhimurium* strains TA100, TA1535, TA98, TA1537, and with activation in TA97.

Reference: Zeiger, E. et al. (1992). Environ. Mol. Mutagen., 19(Suppl. 21):2-141.

Haworth, S. et al., (1983). Environ. Mutagen., 6(Suppl. 1):3-142.

Waleh, N. S. et al. (1982). Mutat. Res., 97:247-256.

Reliability: High because a scientifically defensible or guideline method was used.

Additional References for *In vitro* Bacterial Reverse Mutation Studies:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

DuPont Co. (1978). Unpublished Data, Haskell Laboratory Report No. 751-78, "Mutagenic Activity in the *Salmonella*/Microsome Assay" (December 19).

DuPont Co. (1991). Unpublished Data, Haskell Laboratory Report No. 277-91, "Mutagenicity Testing of 2-Methyl-3-Butenenitrile in the *Salmonella typhimurium* Plate Incorporation Assay" (May 31).

DuPont Co. (1980). Unpublished Data, Haskell Laboratory Report No. 425-80, "Mutagenicity Evaluation in *Salmonella typhimurium*" (June 17).

Type: ***In vitro* Clastogenicity Studies:** No Data.

Type: ***In vivo* Studies:** No Data.

Appendix B

12 February 2004

ROBUST SUMMARY FOR 2-PENTENENITRILE

Existing published and unpublished data were collected and scientifically evaluated to determine the best possible study or studies to be summarized for each required endpoint. In the spirit of this voluntary program, other data of equal or lesser quality are not summarized, but are listed as related references at the end of each appropriate section, with a statement to reflect the reason why these studies were not summarized.

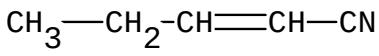
1.0 Substance Information

CAS Number: 13284-42-9 (2-Pentenenitrile)
25899-50-7 (cis-2-Pentenenitrile)
26294-98-4 (trans-2-Pentenenitrile)

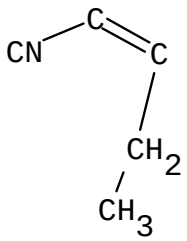
Chemical Name: 2-Pentenenitrile

Structural Formula:

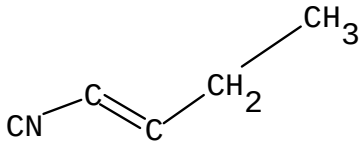
2-Pentenenitrile



cis-2-Pentenenitrile



trans-2-Pentenenitrile



Other Names:

<u>2-Pentenenitrile</u>

Pent-2-enenitrile
1-Cyano-1-butene

<u>cis-2-Pentenenitrile</u>

cis-2-Pentenonitrile
(Z)-Pent-2-enenitrile
2-Pentenenitrile, (Z)-
2-Pentenenitrile, (2Z)
2 PN-HP
cis-1-Cyano-1-butene
cis-1-butenyl cyanide

trans-2-Pentenenitrile

(E)-Pent-2-enenitrile
2-Pentenenitrile, (2E)-
2-Pentenenitrile, (E)-
trans-1-Butenyl cyanide
trans-2-Pentenonitrile

Exposure Limits: 0.3 ppm, 8- and 12-hour TWA; skin: DuPont Acceptable Exposure Limit (AEL) (cis-2-pentenenitrile)

2.0 Physical/Chemical Properties

2.1 Melting Point

Value:	-46.66°C
Decomposition:	No Data
Sublimation:	No Data
Pressure:	760 mm Hg
Method:	Modeled. MPBPWIN, v.1.4, module of EPIWIN 3.05 (Syracuse Research Corporation). MPBPWIN estimates melting point by two different methods. The first is an adaptation of the Joback group contribution method for melting point (Joback, 1982; Reid et al., 1987) and the second is a simple Gold and Ogle method suggested by Lyman, 1985.
GLP:	Not Applicable
Reference:	Joback, K. G. (1982). A Unified Approach to Physical Property Estimation Using Multivariate Statistical Techniques. Stevens Institute of Technology, submitted to the Dept. of Chem. Eng. For M.S. Degree at the Massachusetts Institute of Technology in June 1984 (see also: Reid et al., 1987). Reid, R. C. et al. (1987). The Properties of Gases and Liquids, 4th edition, Chapter 2, Mc-Graw-Hill, Inc., NY. Lyman, W. J. (1985). In: Environmental Exposure From Chemicals, Volume I, Chapter 2, Neely, W. B. and G. E. Blau (eds.), CRC Press, Inc., Boca Raton, FL.
Reliability:	Estimated value based on accepted model.

SUPPORTING DATA: 1-PENTENENITRILE

Value:	-96°C
Decomposition:	No Data

Sublimation:	No Data
Pressure:	No Data
Method:	No Data
GLP:	No Data
Reference:	Lievens (1924). Bull. Soc. Chim. Belg., 22:127 (Beilstein Database, accessed June 17, 2003).
	Timmermans (1927). Bull. Soc. Chim. Belg., 36:507 (Beilstein Database, accessed June 17, 2003).
	Timmermans and Delcourt (1934). J. Chim. Phys. Phys. Chim. Biol., 31:110 (Beilstein Database, accessed June 17, 2003).
	Joutkovsky (1934). Bull. Soc. Chim. Belg., 43:401 (Beilstein Database, accessed June 17, 2003).
	Witschonke (1954). Anal. Chem., 26:562 (Beilstein Database, accessed June 17, 2003
Reliability:	Not assignable because limited study information was available.

SUPPORTING DATA: 9-OCTADECENENTRILE

Value:	-1°C
Decomposition:	No Data
Sublimation:	No Data
Pressure:	No Data
Method:	No Data
GLP:	Unknown
Reference:	Weast, R.C. (ed.) (1979). Handbook of Chemistry and Physics, 60th ed., p. C-404, CRC Press Inc., Boca Raton, Florida.
Reliability:	Not assignable because limited study information was available.

Additional References for Melting Point: None Found.

2.2 Boiling Point

Value:	127°C
Decomposition:	No Data
Pressure:	No Data
Method:	No Data
GLP:	Unknown
Reference:	DuPont Co. (1998). Material Safety Data Sheet No. 6035CR

	(September 18).
Reliability:	Not assignable because limited study information was available.

Additional Reference for Boiling Point:

DuPont Co. (1981). DuPont Commodity Distribution Sheet.

2.3 Density

Value:	0.82
Temperature:	20°C
Method:	No Data
GLP:	Unknown
Results:	No additional data.
Reference:	DuPont Co. (1998). Material Safety Data Sheet No. 6035CR (September 18).
Reliability:	Not assignable because limited study information was available.

Additional Reference for Density:

DuPont Co. (1981). DuPont Commodity Distribution Sheet.

2.4 Vapor Pressure

Value:	4.05 mm Hg
Temperature:	25°C
Decomposition:	No Data
Method:	Estimated using the mean of Antoine & Grain methods.
GLP:	Not Applicable
Reference:	SRC MPBPWIN v1.40 in EPIWIN v3.05.

Syracuse Research Corporation (MPBPWIN) program estimates the boiling point (at 760 mm Hg), melting point, and vapor pressure of organic compounds. The vapor pressure is estimated using the mean of the Antoine and Grain methods. A description of the methodology is detailed in:

Antoine Method: Lyman, W. J. et al. (1990). Handbook of Chemical Property Estimation Methods, Chapter 14, American Chemical Society, Washington, DC.

Modified Grain Method: Lyman, W. J. (1985). In: Environmental Exposure From Chemicals, Volume I,

Chapter 2, Neely, W. B. and G. E. Blau (eds.), CRC Press, Inc., Boca Raton, FL.

Reliability: Estimated value based on accepted model.

Additional References for Vapor Pressure:

DuPont Co. (1981). DuPont Commodity Distribution Sheet.

DuPont Co. (1998). Material Safety Data Sheet No. 6035CR (September 18).

2.5 Partition Coefficient (log Kow)

Value: 1.11
Temperature: Not Applicable
Method: Modeled. The KOWWIN computer program, version 1.66 from Syracuse Research Corporation, calculates the log octanol/water partition coefficient (log Kow) of organic chemicals using an atom/fragment contribution method.
GLP: Not Applicable
Reference: The methodology is described in the following journal article:

Meylan, W. M. and P. H. Howard (1995). J. Pharm. Sci., 84:83-92.

Reliability: Estimated value based on accepted model.

Additional References for Partition Coefficient (log Kow): None Found.

2.6 Water Solubility

Value: 7472 mg/L
Temperature: 22.5°C
pH/pKa: No Data
Method: Water solubility of the test substance was estimated by determining the total organic carbon in water to which an amount of test substance in excess of the water solubility had been added. Samples were tested for their solubility in water after 48 and 96 hours of continuous mixing. Approximately 200 mg of the test substance, prepared in quadruplicate replicates, was added to 20 mL of deionized water in a glass test vessel with a Teflon®-coated screw cap. Two of the replicates were mixed end-over-end for 48 hours and then analyzed. The other 2 replicates were mixed end-over-end for 96 hours before analysis. Prior to analysis, the test vessels were allowed to stand for 1 hour. The upper 5 mL layer of solution was removed by pipette and discarded to

	eliminate test substance at the top of the test vessel. Samples were analyzed for total carbon content via an analyzer with an autosampler attachment. Water solubility was determined by assuming that the test material contained 100% test substances.
GLP:	No
Reference:	DuPont Co. (2002). Unpublished Data, Report No. EMSER 005-02, "Estimated Water Solubility of cis-2-Pentenenitrile" (January 24).
Reliability:	Medium because a suboptimal study design was used.

Value:	7930 mg/L
Temperature:	25°C
pH/pKa:	No Data
Method:	Modeled
GLP:	Not Applicable
Reference:	WsKow v1.40 EPIWIN v3.05 (SRC Database).
	WsKow estimated the water solubility (Wsol) of an organic compound using the compound's log octanol-water partition coefficient (log Kow). The following journal article describes the estimation methodology:
	Meylan, W. M. et al. (1996). Environ. Toxicol. Chem., 15:100-106.
Reliability:	Estimated value based on accepted model.

Additional References for Water Solubility:

DuPont Co. (1981). DuPont Commodity Distribution Sheet.

DuPont Co. (1998). Material Safety Data Sheet No. 6035CR (September 18).

2.7 Flash Point

Value:	26°C
Method:	Closed cup
GLP:	Unknown
Reference:	DuPont Co. (1998). Material Safety Data Sheet No. 6035CR (September 18).
Reliability:	Not assignable because limited study information was available.

Additional Reference for Flash Point:

DuPont Co. (1981). DuPont Commodity Distribution Sheet.

2.8 Flammability

Results:	Flammable
Method:	No Data
GLP:	Unknown
Reference:	DuPont Co. (1998). Material Safety Data Sheet No. 6035CR (September 18).
Reliability:	Not assignable because limited study information was available.

Additional Reference for Flammability:

DuPont Co. (1981). DuPont Commodity Distribution Sheet.

3.0 Environmental Fate

3.1 Photodegradation

Concentration:	Not Applicable
Temperature:	Not Applicable
Direct Photolysis:	Using the absorption spectrum of acetonitrile as an analog example, the nitrile group does not absorp significantly above 200 nm: absorbance at 200 nm = 0.04 absorbance at 210 nm = 0.03 absorbance at 220 nm = 0.01 absorbance at 254 nm = 0.005. Harris (1990) also reported that ethylene, an analog for C=C in the unsaturated mononitriles, has no significant absorption above 290 nm. Therefore, the mononitrile category is expected to lack significant absorptivity above 290 nm and will not be subject to direct photolysis.
Indirect Photolysis:	In the vapor phase, cis 2-pentenenitrile is estimated to have an atmospheric half-life of 1.59 days due to hydroxyl radical oxidation and a half-life of 40.3 days due to reactions with ozone. The two reactions result in an estimated atmospheric half-life of 1.54 days for vapor phase material. In the vapor phase, trans 2-pentenenitrile is estimated to have an atmospheric half-life of 1.4 days due to hydroxyl radical oxidation and a half-life of 20.1 days due to reactions with ozone. The two reactions result in an estimated atmospheric half-life of 1.31 days for vapor phase material.

Breakdown Products:	Not Applicable
Method:	The AOP Program, version 1.90 from Syracuse Research Corporation, estimates the Atmospheric Oxidation Potential. The AOP program estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The methodology used by the Atmospheric Oxidation Program is based upon the structure-activity relationship (SAR) methods developed by Dr. Roger Atkinson and co-workers (Atkinson et al., 1987; 1995; 1996; 1984). The rate constant for the reaction of 2-pentenenitrile vapor with photochemically generated hydroxyl radicals in the atmosphere is estimated to be $1.0x10^{-11}$ cm^3/molecule-sec for the cis isomer and $1.1x10^{-11}$ cm^3/molecule-sec for the trans isomer at 25°C (SRC AOPWin v1.90). These values correspond to respective half-lives of 1.6 days and 1.4 days, assuming a 24 hour day and an ambient hydroxyl radical concentration of $0.5x10^6$ molecules/cm^3.
GLP:	Not Applicable
Reference:	Atkinson, R. et al. (1987). Intern. J. Chem. Kinet., 19 :799-828. Atkinson, R. et al. (1995). Atmos. Environ., 29:1685-1695. Atkinson, R. et al. (1996). Environ. Sci. Technol., 30:329-334. Atkinson, R. et al. (1984). Chem. Rev., 84:437-470. Harris, J. C. (1990). Rate of Aqueous Photolysis, Chapter 8, In: Lyman, W. J. et al. (eds.). Handbook of Chemical Property Estimation Methods, American Chemical Society, Washington, DC. The following journal article describes the AOP Program: Meylan, W. M. and P. H. Howard (1993). Chemosphere, 26:2293-9229.
Reliability:	Estimated value based on accepted model.

Additional References for Photodegradation: None Found.

3.2 Stability in Water

Concentration:	Not Applicable
Half-life:	The Henry's Law constant for 2-pentenenitrile is estimated to be 2.28×10^{-4} atm-m^3/mole (SRC HENRYWIN v3.10 in EPIWIN v3.05) from its estimated vapor pressure of 4.05 mm Hg (SRC MPBPWIN v1.40 in EPIWIN v3.05, mean of Antoine & Grain Methods) and water solubility of 7930 mg/L (WsKow v1.40 EPIWIN v 3.05). The estimated volatilization half-life from a model river (1 m deep, flowing 1 m/sec, wind velocity of 5 m/sec) is approximately 3.2 hours. The estimated volatilization half-life from a model lake (1 m deep, flowing 0.05 m/sec, wind velocity of 0.5 m/sec) is approximately 4.6 days (EPIWIN v3.05).
% Hydrolyzed:	Not Applicable
Method:	Modeled. The WVOL program estimates the volatilization half-lives from a model river and lake using the methodology from Lyman et al., 1990 (adsorption to suspended solids and sediments is ignored). The user can input an experimental water solubility, vapor pressure, or Henry's Law constant or EPI will automatically estimate a Henry's Law Constant from SRC's Henry program for this calculation. WsKow estimates the water solubility (Wsol) of an organic compound using the compounds log octanol/water partition coefficient (log Kow).
GLP:	Not Applicable
Reference:	Lyman, W. J. et al. (1990). The Handbook of Chemical Property Estimation Methods, American Chemical Society, Washington DC. The following journal article describes the estimation methodology: Meylan, W. M. et al. (1996). Environ. Toxicol. Chem., 15:100-106.
Reliability:	Estimated value based on accepted model.

Additional References for Stability in Water: None Found.

3.3 Transport (Fugacity)

Media:	Air, Water, Soil, and Sediments	
Distributions:	Air:	10.8%
	Water:	46.3 %
	Soil:	42.8%

	Sediments:	0.1%
Half-life:	Air:	36.6 hours
	Water:	360 hours
	Soil:	720 hours
	Sediment:	3240 hours
Adsorption Coefficient:	Not Applicable	
Desorption:	Not Applicable	
Volatility:	Not Applicable	
Method:	Calculated according to Mackay, Level III, Syracuse Research Corporation EPIWIN version 3.05. Emissions (1000 kg/hr) to air, water, and soil compartments using standard EPA model defaults with BIOWIN half-life factors of water, 1; soil, 2; and sediments, 9.	
	Data Used: Molecular Weight: 81.12 Chemical Name: 2-Pentenenitrile Henry's Law Constant: 2.28×10^{-4} atm-m^3/mole (HenryWin Program) Vapor Pressure: 4.05 mm Hg (MPBPWIN v1.40) Log Kow: 1.11 (KowWin Program) Soil Koc: 5.28 (Log Kow estimate)	
GLP:	Not Applicable	
Reference:	Syracuse Research Corporation EPIWIN v3.05 contains a Level III fugacity model. The methodology and programming approach were developed by Dr. Donald MacKay and coworkers and are detailed in: Mackay, D. (1991). Multimedia Environmental Models: The Fugacity Approach, pp. 67-183, Lewis Publishers, CRC Press. Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1618-1626. Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1627-1637.	
Reliability:	Estimated value based on accepted model.	

Additional References for Transport (Fugacity): None Found.

3.4 Biodegradation:

Value:	3% after 28 days (Not Readily Biodegradable)
Breakdown Products:	No Data
Method:	The procedure used in the test were based on the recommendations of the following guidelines: OECD Guideline 301B. The biodegradability of cis-2-pentenenitrile was tested using the Modified Sturm test. Biodegradability was measured as CO_2 evolution. A test substance is considered "Readily Biodegradable" if it demonstrates a "pass level" of 60% biodegradability within a 10-day window after exceeding the 10% level of biodegradability. A test substance is considered "Ultimately Biodegradable" if it demonstrates a "pass level" of 60% biodegradability, but not within a 10-day window after exceeding the 10% level of biodegradability. cis-2-Pentenenitrile reached a peak of 3% biodegradability at day 28, and therefore is regarded as not "Readily Biodegradable." Cis-2-Pentenenitrile was not inhibitory to microorganisms in the inoculum.
GLP:	No
Reference:	DuPont Co. (2001). Unpublished Data, Report No. EMSE-073-01, "Biodegradability of cis-2-Pentenenitrile using the Modified Sturm Test (OECD 301B)" (December 17).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Biodegradation: None Found.

3.5 Bioconcentration

Value:	BCF=1.44. This BCF value suggests that bioconcentration potential in aquatic organisms is low.
Method:	The bioconcentration factor is calculated by Syracuse Research Corporation's BCFWIN Computer Program, version 2.14, which utilizes a linear regression based on the log Kow for the compound.
GLP:	Not Applicable

Reference:	The estimation methodology used by BCFWIN is described in the following document prepared for the U.S. Environmental Protection Agency (OPPT): "Improved Method for Estimating Bioconcentration Factor (BCF) from Octanol-Water Partition Coefficient," SRC TR-97-006 (2^{nd} Update), July 22, 1997; prepared for Robert S. Boethling, EPA-OPPT, Washington, DC, Contract No. 68-D5-0012; prepared by William M. Meylan, Philip H. Howard, Dallas Aronson, Heather Printup, and Sybil Gouchie, Syracuse Research Corp., Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212.
Reliability:	Estimated value based on accepted model.

Additional References for Bioconcentration: None Found.

4.0 Ecotoxicity

4.1 Acute Toxicity to Fish:

Type:	**96-hour LC_{50}**
Species:	*Pimephales promelas* (fathead minnow)
Value:	316 mg/L (95% confidence interval, 100-1000 mg/L)
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used that was consistent with OECD Guideline 203, with the following exceptions: 10x dose spacing, 4 test concentrations, and nominal test concentrations were reported. The acute toxicity to fathead minnows was determined in an unaerated, 96-hour static test. The nominal concentrations of cis-2-pentenenitrile used were 0, 1, 10, 100, and 1000 mg/L at a mean temperature of 21.3°C. One test chamber was used per test concentration with 10 test organisms in each chamber. Analysis of the test and control solution samples for dissolved oxygen and pH were made at test initiation (0 hours) and test completion (96 hours).
GLP:	No
Test Substance:	cis-2-Pentenenitrile, purity 98.77%
Results:	Based on visual observations, the water control and all test concentrations were clear and colorless at test start. No precipitate was observed. All water quality parameters were within acceptable limits during the exposure. At test initiation (0 hours), dissolved oxygen and pH ranged from 8.8-8.9 and 7.1-7.5, respectively. Dissolved oxygen and pH

ranged from 6.0-6.5 and 7.2-7.3, respectively, at test completion (96 hours) for all concentrations except 1000 mg/L. Dissolved oxygen and pH for the 1000 mg/L concentration were measured at 24 hours due to total mortality, and were 5.4 and 7.2, respectively.

Exposure of fathead minnows to 1, 10, 100, and 1000 mg/L cis-2-pentenenitrile resulted in 0, 0, 0, and 100% mortality, respectively, at the end of 96 hours. No mortality or sublethal effects were observed in the control organisms.

Reference: DuPont Co. (2000). Unpublished Data, Haskell Laboratory Report No. DuPont-5277, "Static, Acute, 96-Hour Screening Test to *Pimephales promelas*" (December 20).

Reliability: Medium because a suboptimal study design was used (nominal test concentrations).

Type: **96-hour LC_{50}**
Species: Fathead minnow
Value: 474 µg/L; log Kow = 1.11
Method: Modeled
GLP: Not Applicable
Test Substance: 2-Pentenenitrile
Results: No additional data.
Reference: Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability: Estimated value based on accepted model.

SUPPORTING DATA: ACRYLONITIRLE

Type: **96-hour LC_{50}**
Species: *Pimephales promelas* (fathead minnow)
Value: 34,000 µg/L (confidence limits, 28,000-39,000 µg/L)
Method: No specific test guideline was reported. Juvenile fish (1.5-2 month) were used. A concurrent control was tested. Temperature, hardness, alkalinity, dissolved oxygen, pH, and conductivity of the water were reported.
GLP: Unknown
Test Substance: Acrylonitrile, purity >99.9%
Results: The 48-hour LC_{50} was 35,000 µg/L. The following water parameters were reported: temperature of 21.7-22.8°C, hardness of 94-108 mg/L $CaCO_3$, alkalinity of 92-111 mg/L

$CaCO_3$, dissolved O_2 of 4.9-8.5 mg/L, pH of 7.3-8.2, and conductivity of 268-312 μmhos/cm.

Reference: Sabourin, T. D. (1987). Battelle-Columbus Laboratories, Columbus, OH (AQUIRE- 0142088 and 0142089).

Reliability: High because a scientifically defensible or guideline method was used.

Type: **96-hour LC_{50}**

Species: *Pimephales promelas* (fathead minnows)
Lempomis macrochirus (bluegills)
Lebistes reticulatus (guppies)

Value: Fathead minnows (hard water): 14.3 mg/L
Fathead minnows (soft water): 18.1 mg/L
Bluegills (soft water): 11.8 mg/L
Guppies (soft water): 33.5 mg/L

Method: *Acute Toxicity Bioassay*

Bioassays for acute toxicity were conducted by the method recommended by the Subcommittee on Toxicity, Federation of Sewage and Industrial Wastes Association.

Fathead minnows were 2-2.5 inches long and weighed approximately 1.5 g each. Bluegills were 1.5-2 inches long and weighed approximately 2 g each. Guppies were 1 inch long and weighed approximately 0.1 g each.

The characteristics of the dilution waters were as follows:

	Dissolved Oxygen	pH	Alkalinity ppm	Acidity ppm	Hardness ppm
Soft	8.0	7.4	16	2	20
Hard	8.0	8.2	320	0	380

All bioassays were conducted in a constant temperature room at 25°C.

The test substance was water soluble, and was added directly to the selected dilution water in the test aquaria, as a 1% water solution. Test concentrations were prepared using quarter points on a logarithmic scale. In all test concentrations, analyses were made for DO, pH, alkalinity, acidity, and versenate hardness, both initially and after fish mortality or at the end of the test periods. In addition, analyses for cyanides and ammonia were made initially and at the end of 4, 24, 48, and 94-hour periods. The TKN value for 48-hour samples was obtained on the eluate from a cation

exchange column.

Ten fish were added to each test concentration and the bioassays conducted for a 96-hour period. Solutions were not renewed during the test period. Twenty-four, 48, and 96-hour LC_{50} values were computed by straight-line graphical interpolation on semi-logarithmic graph paper.

Chronic Toxicity Bioassay

The chronic bioassays were conducted in a continuous flow apparatus constructed for automatic renewal of test solution over a definite time period. A feed solution containing acrylonitrile (100 to 1000 mg/L) was continuously fed by chemical metering pumps into mixing vessels containing metered quantities of dilution water. After mixing, the test solutions were fed through 5 gallon glass bottles containing 10 test fish in 10 L of solution, and thence to the drain. Solutions in the test aquaria were renewed every 100 minutes, and the feed solution at alternate 8- and 16-hour periods. Fathead minnows were the test fish and the dilution water was soft, comparable to those used in the acute toxicity bioassay. The temperature was maintained at 25°C. Four concentrations and a control were tested simultaneously. The tests were conducted for 30 days or until there were no additional fish mortality after a 10-day period of exposure. The test fish were fed daily either with live *Daphnia* or a ground dry fish food.

GLP: Unknown

Test Substance: Acrylonitrile, purity 98-100%

Results: *Acute Toxicity Bioassay*

The following results were reported:

Fish	Dilution water	24-hour LC_{50}	48-hour LC_{50}
Fathead minnows	Hard	32.7	16.7
Fathead minnows	Soft	34.3	21.5
Bluegills	Soft	25.5	14.3
Guppies	Soft	44.6	33.5

Apparently, changes in water quality characteristics (within the range studied) did not greatly influence the toxicity. Acrylonitrile was slightly more toxic in hard water.

The nitrile, within the range of concentrations tested, did not greatly alter characteristics of the dilution water. The chemical analyses for cyanides and ammonia indicated little or no formation of these substances (by breakdown of a nitrile) during the test period. Ammonia results were very erratic with formation of small amounts, probably from waste products of fish, but with no clear indication of formation by breakdown of any of the nitriles.

Chronic Toxicity Bioassay:

The following results were reported:

Exposure Time	TLm (mg/L)
24 hours	33.5
48 hours	14.8
72 hours	11.1
96 hours	10.1
5 days	8.1
10 days	6.9
15 days	5.2
20 days	4.2
25 days	3.5
30 days	2.6

The continuous flow values for acrylonitrile are the average of 5 replicate experiments with 50 fish exposed to each of 7 different concentrations for up to 30-day periods. Thirty-day TLm values ranged from 2.4 to 3.0 mg/L with an

average of 2.6 mg/L. A very definite chronic or accumulative effect was shown for this compound with mortality continuing throughout the 30-day period of exposure. If 30-day TLm values are compared with those for 48 or 96 hours, there is a 4-6-fold increase in toxicity during this period. If results in static (non-renewed) and continuous flow solutions are compared, it was observed that 24-hour TLm values were comparable. However, after this initial period, the toxicity was greater in renewed solutions, which would indicate some loss of acrylonitrile through volatility, chemical change, or bio-oxidation, in the non-renewed solutions.

There was no fish mortality from acrylonitrile in the highest test concentrations (50 mg/L) in less than 10 hours. The first visible fish reaction to acrylonitrile was an extreme darkening of the skin. In some of the moderate to low test concentrations fish would live for 10-20 days without any observable effect, then would suddenly turn dark, almost black, and succumb within 1 to 3 days.

Reference: Henderson, C. et al. (1961). Proc. 15th Ind. Waste Conf., 65(2):120-130.

Reliability: High because a scientifically defensible or guideline method was used.

Type: **96-hour LC_{50}**

Species: *Lepomis macrochirus* Rafinesque (bluegill sunfish)

Value: Static test: 23.6 ppm (95% confidence limits, 22.7-24.4 ppm)
Flow-through test: 9.3 ppm (95% confidence limits, 8.7-10.0 ppm)

Method: The tests were performed according to guidelines given by the EPA (EPA-660/3-75-009).

Static Toxicity Test

In the static toxicity test, fish were exposed to a minimum of concentrations, using 19-L glass, wide-mouth jars as exposure chambers and no aeration. The test concentrations (not reported) were prepared in duplicate, and the fish were distributed among the exposure chambers by stratified random assortment (10 fish per duplicate, 20 fish per concentration). Range-finding tests were conducted to determine the proper concentrations to use in the definitive test. The fish were checked several times daily. Dead fish

were removed as they occurred and mortality cumulated every 24 hours; the test was terminated after 96 hours. The pH of the test solutions was measured at the beginning and end of the test. Dissolved oxygen concentration was measured daily in each test solution, and the quantity of toxicant in the test solutions was measured at the beginning of the test.

The test solutions were maintained at 22±1°C by partially submerging the exposure chambers in a thermostatically controlled water bath. The temperature of the bath was monitored hourly. Periodically, the temperature of the test solutions was checked with a glass thermometer.

Flow-Through Toxicity Test

The flow-through toxicity tests were conducted with 2 exposure schedules. In the first, the exposure period was 96 hours, and mortality was recorded every 24 hours. In the second, the exposure period was 24 hours, and mortality was recorded at 1, 2, 4, 8, 16, and 24 hours.

Glass exposure chambers (15 L), with drains set to dispose water in excess of 11 L from the chambers, were used. Water and toxicant were delivered to a mixing-distributing vessel, which in turn delivered the mixture to 2 exposure chambers. The rate of water delivery was 50 mL/min per chamber, or 6.54 tank volumes per 24 hours.

A stock solution of the test substance was prepared and metered into the mixing-distributing vessel. To minimize volatilization, all the organic compounds and ammonia were metered with Sage syringe pumps equipped with 1 or more gas-tight Hamilton syringes. For the diluent water, a system developed at SRI that incorporates a constant head reservoir and flow meters to maintain the appropriate flow rates was used.

A stock solution of the test substance was prepared and metered into the mixing-distributing vessel. To minimize volatilization, all the organic compounds and ammonia were metered with Sage syringe pumps equipped with 1 or more gas-tight Hamilton syringes. For the diluent water, a system developed at SRI that incorporates a constant head reservoir and flow meters to maintain the appropriate flow rates was used.

In each test, a minimum of 4 test concentrations plus a control group in duplicate were used, and the fish were distributed among the exposure chambers by stratified random assortment. The water and toxicant flows were checked daily and adjusted when necessary. The pH and dissolved oxygen and toxicant concentrations of the test solutions were also determined at the beginning and end of each test.

GLP: Unknown

Test Substance: Acrylonitrile, purity practical grade

Results: Additional LC_{50} values are listed in the table below.

Exposure Time (hours)	Static LC_{50} (ppm)	Flow-Through LC_{50} (ppm)
1	-[a]	1480
2	-	618
4	-	120.
8	-	58
16	-	30.8
24	30.3	18.7[b] >26[c]
48	25.7	18
72	23.6	14.7

[a] No data
[b] From the 24-hour flow-through toxicity test.
[c] From the 96-hour flow-through toxicity test.

The nominal and measured concentrations used in the static and flow-through tests differed by up to a factor of 10. Water quality parameters, such as temperature, pH, and dissolved oxygen were monitored. In all tests, the water temperature was 22±1°C. In the flow-through test, preparation of the diluent water enabled the authors to maintain the dissolved oxygen concentrations above 7.5 ppm or 85% saturation. The pH of the test solution varied depending on the concentration of the compound in the exposure chambers. The pH ranged from 6-8 in the flow-through tests and from 6-9 in the static test.

Reference: Bailey, H. C. et al. (1985). In: Bahner, R. C. and D. J. Hansen (eds.) (1985). Aquatic Toxicology and Hazard Assessment, 8th Symposium, ASTM STP 891, pp. 193-212, Philadelphia, PA.

Reliability: High because a scientifically defensible or guideline method was used.

Type:	**96-hour LC_{50}**
Species:	Fathead minnow
Value:	414 μg/L; log Kow = 0.21
Method:	Modeled
GLP:	Not Applicable
Test Substance:	2-Propenenitrile
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Fish: None Found.

4.2 Acute Toxicity to Invertebrates:

Type:	**48-hour EC_{50}**
Species:	*Daphnia magna* (water flea)
Value:	114 mg/L (95% confidence interval, 77-276 mg/L)
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used that was consistent with OECD Guideline 202, with the following exceptions: 10x dose spacing, 4 test concentrations, nominal test concentrations were reported, and 1 replicate per test concentration was performed. The acute toxicity to *Daphnia magna* (less than 24 hours old) was determined in an unaerated, 48-hour static test. The nominal concentrations of cis-2-pentenenitrile used were 0, 1, 10, 100, and 1000 mg/L at a mean temperature of 20.1°C. One test chamber was used per test concentration with 10 test organisms in each chamber. Analysis of the test and control solution samples for dissolved oxygen and pH were made at test initiation (0 hours) and test completion (48 hours).
GLP:	No
Test Substance:	cis-2-Pentenenitrile, purity 98.77%
Results:	Based on visual observations, the water control and all test concentrations were clear and colorless at test start. No precipitate was observed. All water quality parameters were within acceptable limits during the exposure. At test

initiation (0 hours), dissolved oxygen was 8.6 and pH ranged 6.9-7.8. At test completion, dissolved oxygen and pH ranged from 7.8-7.9 and 7.6-8.0, respectively.

Exposure of daphnids to 1, 10, 100, and 1000 mg/L cis-2-pentenenitrile resulted in 0, 10, 40, and 100% immobility, respectively, at the end of 48 hours. No immobility or sublethal effects were observed in the control organisms.

Reference: DuPont Co. (2000). Unpublished Data, Haskell Laboratory Report No. DuPont-5278, "Static, Acute, 48-Hour Screening Test to *Daphnia magna*" (December 20).

Reliability: Medium because a suboptimal study design was used (nominal test concentrations).

SUPPORTING DATA: ACRYLONITIRLE

Type: 48-hour LC_{50}
14- and 21-day Chronic Toxicity

Species: *Daphnia magna*

Value: 48-hour LC_{50}: 10 mg/L
14- and 21-day ChV (reproduction): 0.71 mg/L
14- and 21-day ChV (survival): >2.8 mg/L

Method: The static acute toxicity test was conducted according to the requirements of the OECD Guidelines for Testing Chemicals. The test was conducted without food in the dilution water, and the chemical concentrations were not measured. The test solution was changed once every day. The test temperature was 24±1°C. Mortality was measured at 48 hours and a 48-hour LC_{50} was calculated. The parameters of water quality were measured at the beginning and the end of the test.

The chronic renewal test was performed following OECD Guidelines for Testing Chemicals. All tests were 1 generation, 21 days long, and were started with *Daphnia* less than 24 hours of age. There were 4 treatment groups and a control group, 4 beakers per group, and 3 *Daphnia* per 150 mL beaker. Each beaker contained 100 mL of test solution. The test solution was renewed once per day. The photoperiod was 16 hours light and 8 hours dark, and the light density was 2000 Lux. The test temperature was the same as that in the acute test. The test concentrations were 0, 0.25, 0.5, 1, and 2 mg/L. The *Daphnia were* fed with *Selenastrum capricornutum*. Survival and reproduction were measured every day.

For the acute test, the probit method was used to calculate the LC_{50}. For the chronic tests, the reproduction data did not satisfy the normal distribution hypothesis, and every group had 4 beakers. So Steel's Many-One Rank test was used to calculate the NOEC, LOEC, and ACR (Acute Chronic Ratio) for reproduction. Fisher's Exact method was used to arrive at NOEC, LOEC, and ACR for survival. ACR is defined as the ratio of 48-hour LC_{50} and ChV (Chronic Value), where ChV is the geometric mean value of NOEC and LOEC.

GLP: Unknown

Test Substance: 2-Propenenitrile, purity not reported

Results: The deviation of pH was less than 0.3, and the concentrations of dissolved oxygen in the test solution were higher than 5 mg/L, so pH and DO measurements satisfied the requirements of OECD test guideline. The survival percentage of control group was 100% at the end of the test. The mean number of young per female *Daphnia* in the control group was 231. Therefore, the survival and reproduction results were both in accordance with the requirements of the OECD test guideline.

The sensitivity of reproduction and survival in the 14-day test was the same as in the 21-day test. The following table lists the survival and reproduction (number of neonates per female) during the 14- and 21-day tests.

Concentration (mg/L)	Survival (%)		Reproduction	
	21-day	14-day	21-day	14-day
0	100	100	231	140
0.25	83	92	230	131
0.5	100	100	210	133
1.0	92	100	209	130
2.0	75	92	205	126

The following table lists the NOEC, LOEC, CHV, and ACR values for the 14- and 21-day tests.

		14-day	21-day
48-hour LC_{50}		10 mg/L	
Reproduction	NOEC	0.5 mg/L	
	LOEC	1.0 mg/L	
	ChV	0.71 mg/L	
	ACR	16	
Survival	NOEC	2.0 mg/L	
	LOEC	>2.0 mg/L	
	ChV	>2.0 mg/L	
	ACR	<5	

Reference: Tong, Z. et al. (1996). Bull. Environ. Contam. Toxicol., 57:655-659.

Reliability: High because a scientifically defensible or guideline method was used.

Type: **48-hour LC_{50}**

Species: *Daphnia magna*

Value: 7.6 mg/L (confidence limits, 6.2-9.2 mg/L)

Method: Daphnia magna were 24 hours old. Water used to culture the organisms was reconstituted according to the EPA to a total hardness of 173±13 mg/L as $CaCO_3$ and a pH of 8.0±0.2, to improve conditions for test organisms.

Procedures used in the acute toxicity test were based on protocols in EPA's "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians." Diluent water used in these tests was of the same quality as previously described for water used to culture these animals. At the initiation of all tests, the dissolved oxygen concentration of diluent water was greater than 60% of saturation. Temperature of test solutions was maintained at 22±1°C.

Addition of the test material to diluent water varied according to the water solubility of the chemical. A stock solution of the chemical, in distilled water, was prepared and used to provide the desired concentrations for testing if the test material was sufficiently soluble in water. If insoluble at the desired water stock solution concentrations, a stock solution utilizing a co-solvent was prepared. If the chemical was not sufficiently soluble in either distilled water or a co-solvent for the preparation of a stock solution, then the

chemical was added directly to the diluent water. Five to 8 nominal concentrations of each chemical were tested.

Using 1 of the methods described above, the chemical to be tested was added to diluent water in 2 L jars to prepare each test solution. If the chemical was soluble in the diluent water, then the volume of test solution was divided into 3 aliquots n 250 mL beakers to provide triplicate exposures. The remaining control, high, middle, and low test concentrations were used to measure the 0-hour dissolved oxygen concentration and pH of the solutions. Five daphnids were randomly placed in each solution within 30 minutes of the solution preparation. IF the test material was not soluble in the diluent water, the test mixtures were not divided into triplicate test vessels, but were retained in the 2 L mixing jars. Fifteen daphnids were placed directly into the 2 L jars containing diluent water prior to addition of the test material. The tests were also conducted in unreplicated solutions containing 15 daphnids if dividing the solution into triplicate test vessels presented a risk of the loss of the test substance through volatilization or if vapors of the substance posed a high health risk to the investigators. In addition, these vessels were covered with plastic wrap secured with an elastic band.

A negative control consisting of the same dilution water, test conditions, and test organisms, but containing no test substance or co-solvent, was maintained concurrently with each test. When appropriate, a positive (solvent) control was also established consisting of the same dilution water, conditions, and a number of test organisms as in the negative control and containing the highest concentration of the co-solvent present in any test vessel.

During the test, the dissolved oxygen concentration, pH, and temperature of test solutions were measured at the initiation and termination of the toxicity tests in the high, middle, and low test concentrations and controls. These parameters were measured only at the end of an exposure if a potential loss of the test substance existed due to volatilization or unsolubilized test material adhering to instrument probes.

Observations of test populations were made at 24 and 48 hours of exposure and any mortalities were recorded.

	Mortality data were used to calculate the LC_{50} based on the nominal concentration to which the *Daphnia* were exposed at the initiation of the test. The LC_{50} was calculated utilizing a moving average angle method, when possible. When test data did not meet the moving average angle method requirements, the LC_{50}'s were estimated by probit analysis by converting the concentrations to logarithms and percentage mortalities to probits and then calculating a least squares linear regression analysis. Finally, if the data did not permit a probit analysis, then a binomial probability analysis was performed on these data.
GLP:	Unknown
Test Substance:	Acrylonitrile, purity ≥ 80%
Results:	The 24-hour LC_{50} was 13 mg/L and the no discernible effect concentration was 0.78 mg/L. Mortality among *Daphnia* control populations never exceeded 10%. Measurements of water quality characteristics revealed that dissolved oxygen concentrations ranged from 6.5-9.1 mg/L during the 48-hour exposure period. Within any 1 test, the greatest range observed was 6.6-8.1 mg/L. The range of pH values was 7.4-9.4 units.
Reference:	LeBlanc, G. A. (1980). Bull. Environm. Contam. Toxicol., 24:684-691.
Reliability:	High because a scientifically defensible or guideline method was used.
Type:	**48-hour LC_{50}**
Species:	*Daphnia magna*
Value:	10.95 mg/L (95% confidence limits, 9.54-12.56 mg/L)
Method:	The test was conducted on *Daphnia magna*, which were cultured according to the method of Beisinger. Dilution water from a local spring-fed pond was used as culture media and for toxicity tests. The water was relatively hard, averaging 154.5 mg/L of hardness measured as $CaCO_3$ over the period of use. The acute static tests were conducted as described by the EPA. First instar *Daphnia* (12±12 hours old) were used for the test. The test was conducted in duplicate for 48 hours at 22°C in a constant-temperature chamber. All compound concentrations were nominal. The 48-hour EC_{50} value was estimated by the method of Litchfield and Wilcoxon.
GLP:	Unknown
Test Substance:	Acrylonitrile, purity not reported
Results:	Spring water analysis revealed a pH of 7.0-8.2, hardness of 89.5-180 mg/L as $CaCO_3$, alkalinity of 95-156 mg/L as

	$CaCO_3$, and conductivity of 225-325 µmhos/cm.
Reference:	Randall, T. L. and P. V. Knopp (1980). Water Polllut. Control Fed. J., 52(8):2117-2130.
Reliability:	Medium because a suboptimal study design was used (nominal test concentrations).
Type:	**48-hour LC_{50} and 21-day ChV**
Species:	*Daphnia magna* *Artemia salina*
Value:	48-hour LC_{50} *Daphnia magna*: 8.697 mg/L 48-hour LC_{50} *Artemia salina*: 14.34 mg/L 21-day ChV *Daphnia magna*: 0.707 mg/L
Method:	*Acute Toxicity Test*

The test procedures followed the methods recommended by the relevant agencies (APHA, China NEPS, EPA).

Tap water, dechlorinated with activated carbon was used as dilution water was used for the test with *Daphnia magna*. The measured quality parameters of dilution tap water were as follows: pH 7.0±0.5, DO 8.0±0.34 mg/L, COD 1.423±mg/L, and hardness 1.86±0.08 mg/L as $CaCO_3$. The dilution water used in *Artemia salina* acute toxicity test was marine water formulated according to standard methods. The test organisms were allowed to gradually acclimate to dilution water for a minimum of 48 hours.

The tests were static-renewal in which test solutions were totally renewed at 24-hour intervals. Standard conditions were 5 concentrations and a control for each test, except for 6 concentrations for *Daphnia magna*. The tests were under 16:8 (light:dark) photoperiod. Animals were not fed during the tests. Each test was performed twice.

Neonates of *Daphnia magna* and *Artemia salina*, less than 24 hours of age, were tested in 150 mL beakers containing 100 mL test solution at 25±1°C. Each treatment group had 3 replicate beakers and 10 neonates for each beaker.

Chronic Toxicity Test

The 21-day survival-reproduction test using neonates of *Daphnia magna* aged no more than 24 hours were conducted in 150 mL beakers containing 100 mL test solution each at 24±1°C in the static-renewal mode under 16:8 (light:dark) photoperiod. The daphnids were fed with green algae at a

	cell concentration of 5.0 ± 10^6 cells/mL test solution. Test solutions were totally renewed at 24-hour intervals and the reproduction/survival data were recorded each day. The lower chronic limit (LCL), upper chronic limit (UCL), and chronic value (ChV) were derived by analyzing survival rates and reproduction rates of the organisms exposed until the 21st day. According to the raw data distribution, Trimmed Spearman-Katver (TSK) method was employed to calculate the LC_{50}. Data on survival of daphnids were analyzed using Fisher's Exact method. Raw data on reproduction of daphnids were not normally distributed, and should be tested using "Steel's Many One Rank Procedure" according to the EPA.
GLP:	Unknown
Test Substance:	Acrylonitrile, purity analytical grade
Results:	Results of the 21-day test with *Daphnia* demonstrated that less reproduction rates were significantly observed at 1.0 mg/L, but survival rates were not remarkably reduced even at 2.0 mg/L. At the test end, survival rate of 100% and reproduction rate of 231.5±2.0 offspring/adult individual were observed in the control group and satisfied the requirements of tests with this species recommended by the U.S. EPA and China NEPA. LCL and UCL for reproduction was 0.5 and 1.0 mg/L, respectively. Using ChV and ACR equations, the ChV of 0.707 mg/L and ACR of 12.63 mg/L were derived.
Reference:	Tong, Z. et al. (1996). Chemosphere, 32(10):2083-2093.
Reliability:	High because a scientifically defensible or guideline method was used.
Type:	**96-hour LC_{50}**
Species:	*Mysidopsis bahia* (opossum shrimp)
Value:	5810 µg/L (confidence limits, 4650-7250 µg/L)
Method:	No specific test guideline was reported. Water parameters reported include temperature, dissolved oxygen, pH, salinity, and organic carbon.
GLP:	Unknown
Test Substance:	Acrylonitrile, purity >99.9%
Results:	The following water parameters were reported: temperature of 20.9°C, dissolved oxygen of 7.2 mg/L, pH of 8.05, salinity of 32.0 ppt, and organic carbon of 2.8 mg/L.
Reference:	Carr, R. S. (1987). Battelle Ocean Sciences, Druxbury, MA (AQUIRE-0166627).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Toxicity to Invertebrates: None Found.

4.3 Acute Toxicity to Aquatic Plants:

Type:	**72-hour EC_{50}**
Species:	*Selenastrum capricornutum* (green alga)
Value:	263.5 mg/L (95% confidence interval, 86.8-113.4 mg/L)
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used that was consistent with OECD Guideline 201. Algae were exposed to nominal concentrations of 0, 0.01, 0.1, 1, 10, 100, and 1000 mg/L cis-2-pentenenitrile. After addition of algal cells, the test and control flasks were placed on a shaker table in a chamber with a temperature of approximately 25°C. The algae were incubated for 72 hours without medium renewal. Illumination was supplied by cool-white fluorescent tubes (mean light intensity of 7334 lumens/m^3). The shaking speed was 90 revolutions per minute (rpm). The effect was expressed as percent inhibition growth based on healthy cell count (also referred to as cell density) relative to the control.
GLP:	No
Test Substance:	cis-2-Pentenenitrile, purity 98.77%
Results:	Based on visual observations, the control and all test concentration solutions were clear and had no color before addition of algal cells. The pH of the culture medium at study start was 7.45, and the pH of the test solutions at study start was 6.88-7.45, and at test termination was 7.00-7.28. The NOEC was 100 mg/L. The initial control cell culture was 10,000 cells/mL. At 72 hours, the mean control cell culture was 2.86×10^6 cells/mL (286X initial control culture).
Reference:	DuPont Co. (2000). Unpublished Data, Haskell Laboratory Report No. DuPont-5279, "Influence on Growth and Growth Rate of the Green Alga *Selenastrum capricornutum*" (November 29).
Reliability:	Medium because a suboptimal study design was used (nominal test concentrations).

SUPPORTING DATA: Acrylonitrile

Type:	**96-hour IC_{50}**
Species:	*Lemna minor* (duckweed)

Value:	27.08 mg/L
Method:	The culture solution was used as the dilution water to prepare test solutions. During the tests, the test solutions were placed in 500 mL Erlenmeyer flasks covered with cotton stoppers to reduce the volatilization. Concentrations were 0, 6.2, 12.5, 25, and 50 mg/L. Every treatment group had 4 replicate containers. Ten fronds were added to each container and cultured under the same light as that in pre-culturing. The test was performed at 23+2°C. Test solutions were renewed daily. The number of fronds was counted every day for 4 days. In order to eliminate subjective decisions, every frond visibly projecting beyond the edge of the parent frond was counted. As fronds age and die, they lose their pigment and become chlorotic. The chlorotic fronds were not included in the total frond count. The 4-day period was chosen to be comparable to those acute endpoints, avoid overgrowing, and avoid competition by algae. The inhibition percent relative growth rate was selected as the endpoint for biomass response to the toxicant. Concentration (log 10) was plotted against inhibition (%) to determine the 96-hour EC_{50} values. The line of best fit and the concentration corresponding to 50% inhibition were calculated by inverse linear regression. Samples were independent in this study. The data were first tested for normality, and then tested for homogeneity of variance across all concentrations and controls using Bartlett's method. Then Dunnett's procedure was performed on homostatic data to identify the significance difference, and calculate the NOECs and LOECs.
GLP:	Unknown
Test Substance:	2-Propenenitrile, purity not reported
Results:	The data were normally distributed and had homogenous variance. The NOEC and LOEC were 6.2 and 12.5 mg/L, respectively.
Reference:	Tong, Z. and J. Hongjun (1997). Environ. Pollut., 98(2):143-147.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Toxicity to Aquatic Plants: None Found.

5.0 Mammalian Toxicity

5.1 Acute Toxicity

Type:	**Oral ALD**
Species/strain:	Male rats/Crl:CD®BR
Value:	450 mg/kg
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Male rats (1/dose level), approximately 7 weeks old upon arrival, were administered 130, 200, 300, 450, 670, 1000, or 2300 mg/kg cis-2-pentenenitrile dispersed in corn oil via intragastric intubation. Following administration of the test substance, rats were observed for clinical signs of toxicity. Surviving rats were weighed daily and observed daily until signs of toxicity subsided, and then at least 3 times/week throughout a 14-day post-exposure period. Observations for mortality were made daily throughout the study.
GLP:	Yes
Test Substance:	cis-2-Pentenenitrile, purity 98.6%
Results:	Mortality occurred at doses ≥450 mg/kg. Deaths occurred up to 2 days after dosing. The rat dosed at 130 mg/kg exhibited no clinical signs of toxicity. Clinical signs of toxicity observed at lethal doses included hyperactivity, spasms, incoordination (450 mg/kg); lethargy (≥670 mg/kg); and clear ocular discharge (2300 mg/kg). Clinical signs of toxicity observed within the 1st 2 days after dosing at non-lethal doses included hyperactivity, tremors, spasms, and incoordination (200 and 300 mg/kg). Moderate to severe weight losses (up to 20% of initial body weight) were observed up to 2 days after dosing.
Reference:	DuPont Co. (1990). Unpublished Data, Haskell Laboratory Report No. 197-90, "Approximate Lethal Dose (ALD) of 2-Pentenenitrile in Rats" (May 17) (also cited in TSCA fiche OTS0000790).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Oral Toxicity:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

DuPont Co. (1976). Unpublished Data, Haskell Laboratory Report No. 35-76,

"Acute Oral Test" (January 21) (also cited in TSCA fiche OTS0000790 and OTS0571588).

DuPont Co. (1976). Unpublished Data, Haskell Laboratory Report No. 36-76, "Acute Oral Test" (January 21) (also cited in TSCA fiche OTS0000790 and OTS0571643).

DuPont Co. (1975). Unpublished Data, Haskell Laboratory Report No. 409-75, "Acute Oral Test" (July 11) (also cited in TSCA fiche OTS0529919).

Dow Chemical Co. (1983). R & D Report (March 1) (TSCA fiche OTS0537162).

Data from these additional sources were not summarized because the study design was not adequate.

Tanii, H. et al. (1989). Neurotoxicology, 10:157-166.

Tanii, H. et al. (1991). Neuropharmacology, 30(8):887-892 (TOXLINE/1992/44421).

Type:	**Inhalation LC_{50}**
Species/Strain:	Male and female rats/CRL:CD®BR
Exposure Time:	4 hours
Value:	850 ppm (95% confidence interval, 740-970 ppm)
Method:	The procedures used in the test were based on the recommendations of the following guideline: U.S. EPA Health Effects Testing Guidelines 40 CFR.798.1150. Groups of young adult male and female rats (5/sex/dose level), approximately 7 weeks of age upon arrival, were exposed nose-only to 18, 140, 650, 900, or 1200 ppm cis-2-pentenentrile. The male rats weighed 240-299 g, and female rats weighed 175-235 g on the day of exposure. Rats were observed for mortality and clinical signs of toxicity immediately after they were removed from the restrainers following exposure, and during a 14- or 28-day post-exposure period. The 14-day recovery period was extended to 28 days for rats exposed to 140, 650, 900, or 1200 ppm cis-2-pentenenitrile due to the persistence of clinical signs. In addition, body weights were recorded. At the end of the recovery period, all surviving rats were euthanized without patholological evaluation.

During exposure, rats were restrained in perforated, polycarbonate cylinders with conical nose pieces. The restrainers were inserted into the face plate of a 29-L cylindrical glass exposure chamber so that only the nose of each rat protruded into the chamber. Atmospheres of cis-2-pentenenitrile were generated by vaporization of the test substance in a stream of filtered air heated to 100ºC. The test substance was metered into a J-shaped glass tube containing glass beads with an infusion pump. Chilled, filtered dilution air was added to the heated air/test substance mixture. The generation train then fed into a 29-L glass exposure chamber with a baffle positioned in the air stream to aid in the distribution of the test substance within the chamber. The atmospheric concentration of cis-2-pentenenitrile vapor was determined during each exposure. Chamber airflow was set initially for the exposure and maintained at a constant rate throughout the 4-hour exposure. Generation and dilution airflows were set and monitored. Chamber temperature, relative humidity, and oxygen concentration were measured.

The LC_{50} was determined using probit analysis.

GLP: Yes

Test Substance: cis-2-Pentenenitrile, purity 98.6%

Results: The mean total vapor concentrations were 18, 140, 650, 900, and 1200 ppm. During the animal exposures, the total airflow was held constant at 35 L/min and chamber temperatures ranged from 23-28ºC. The relative humidity in the test chamber ranged from 21-58%, and chamber oxygen was 21%. A study of the chamber distribution of vapor was performed. The vapor was considered to be homogeneously distributed at a design concentration of 1000 ppm cis-2-pentenenitrile.

Mortality was 0/10, 0/10, 0/10, 8/10, and 9/10 at 18, 140, 650, 900, and 1200 ppm, respectively. The majority of the rats died within 1-2 days following exposure, with the exception of one female rat exposed at 900 ppm that died 4 days following exposure. There were no sex-related differences in the response of rats in this study based on the lethality.

Clinical signs of toxicity could not be assessed during exposure because the method of restraint prevented clear visual observation of the rats. During exposures, tapping on the chamber elicited a response from all rats. Upon removal

of the rats from restrainers immediately following exposures, clinical observations included nasal and oral discharges. Wet perineum and wet back were also observed, but are a common finding associated with the method of restraint used in this study. Males exposed at 18 ppm showed nasal discharges, and in 1 rat ocular discharge following exposure. No clinical signs were observed in females exposed at this level. Clinical observations that were observed, but were not common in rats exposed at higher concentrations included muscle fasciculation, shut eye(s), moribundity, and exophthalmus. Clinical observations noted in rats following exposure at levels producing lethality (900 or 1200 ppm) included irregular respiration, immobility, and lethargy.

A number of clinical signs that were observed were suggestive of central nervous system effects. These included abnormal gait and mobility, ataxia, hyperreactivity, hypersensitivity, tremors, forward circling, backward circling, splaying of the feet, bobbing of the head, vocalizations, chattering of the teeth, rolling behavior, wobbling behavior, and walking backward. Effects such as abnormal gait, ataxia, circling behavior, head bobbing movement, vocalizations, and tremors were noted in non-lethal levels as low as 140 ppm. Many of these effects appeared reversible within 1-4 days following exposure. However, abnormal gait and mobility, circling behaviors, splaying of the feet, and head bobbing movement were observed throughout the 28-day recovery period in rats exposed at concentrations of 650 ppm or greater. These clinical signs, when present in rats exposed at 140 ppm, were not as persistent as in rats exposed at levels greater than 140 ppm. Other clinical signs noted during the recovery period included alopecia, lung noise, weakness, and stained perineum.

By the 1st day following exposure, most rats exhibited weight loss. Male rats exposed to 18 ppm cis-2-pentenenitrile lost from 2-4% of their initial body weight, while females lost from 0.3-4% of their initial body weight. At higher concentrations (exposures ≥140 ppm), male rats lost from 10-17% and females lost 4-14% of their initial body weight. Generally, all rats gained weight over the recovery period, but surviving rats exposed at all levels experienced some periods of transient body weight loss throughout the recovery period.

Reference:	DuPont Co. (1991). Unpublished Data, Haskell Laboratory Report No. 75-91, "Acute Inhalation Toxicity Study with cis-2-Pentenenitrile in Rats (June 5) (also cited in TSCA fiche OTS0529919).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Inhalation Toxicity:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

DuPont Co. (1976). Unpublished Data, Haskell Laboratory Report No. 42-76, "Acute Inhalation Toxicity" (January 21) (also cited in TSCA fiche OTS0000790).

DuPont Co. (1976). Unpublished Data, Haskell Laboratory Report No. 43-76, "Acute Inhalation Toxicity" (January 21) (also cited in TSCA fiche OTS0000790 and OTS0571587).

U. S. EPA (n.d.). EPSAR 8EHQ-1190-1121 (RTECS/SB2260000).

Data from this additional source were not summarized because the study design was not adequate.

DuPont Co. (1993). Unpublished Data, Haskell Laboratory Report No. 253-93, "Acute Inhalation Neurotoxicity Study in Rats" (December 3).

Type:	**Dermal ALD**
Species/Strain:	Male rabbits/Albino
Exposure Time:	24 hours
Value:	300 mg/kg
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Male rabbits (1/dose level) were clipped free of hair over the back and trunk area and fitted with plastic collars. They were dosed with 60, 90, 130, 200, 300, 450, 670, 1500, 2500, or 4000 mg/kg cis-2-pentenenitrile at 67.7% or 37.4%. The rabbits were treated by applying the test material to gauze pads, then wrapped successively with plastic wrap, stretch gauze and elastic adhesive bandage. Wrappings were removed from surviving animals after 24 hours, and the animals were observed for 14 days.

GLP:	No
Test Substance:	cis-2-pentenenitrile, purity 67.7% or 37.4%
Results:	Deaths occurred at 300 mg/kg and above. All deaths occurred within 5 hours after dosing. Clinical signs at lethal doses included dilated pupils, static ataxia, tremors, prostration, and tonic then clonic convulsions. Clinical signs observed at non-lethal doses included dilated pupils, static ataxia, and tremors. The clinical signs returned to normal in all surviving animals within 24-48 hours.
Reference:	DuPont Co. (1976). Unpublished Data, Haskell Laboratory Report No. 78-76, "Skin Absorption Approximate Lethal Dose (ALD) on Rabbits (February 5) (also cited in TSCA fiche OTS0000790 and OTS0571460)
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Dermal Toxicity:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

DuPont Co. (1983). Unpublished Data, Haskell Laboratory Report No. 65-83, Acute Skin Absorption LD_{50} Test on Rabbits (March 1) (also cited in TSCA fiche OTS0000790).

Dow Chemical Co. (1983). R & D Report (March 1) (TSCA fiche OTS0537162).

Data from this additional source were not summarized because the focus of the study was to determine if the test substance was a Class B poison.

DuPont Co. (1983). Unpublished Data, Haskell Laboratory Report No. 543-83, "Class B Poison Test on Rabbit Skin" (December 7) (also cited in TSCA fiche OTS0529919).

Type:	**Dermal Irritation**
Species/Strain:	Female rabbits/New Zealand White
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. On the day prior to study initiation, the hair of 6 female rabbits was closely clipped to expose the skin from the scapular to the lumbar region of the back. The rabbit's body weights ranged from 2978-3169 g on the day of treatment. Each rabbit was placed into a stock that had been fitted with

	a piece of rubber sheeting. The rabbits remained in the stocks throughout the exposure period and during that time did not have access to food or water. Approximately 0.5 mL of 2-pentenenitrile was applied directly on the test site beneath a gauze square that was held in place with non-irritating tape. The rubber sheeting was then wrapped around the animal and secured with clips to retard evaporation and to keep the test material in contact with the skin without undue pressure. Approximately 24 hours after application of the test substance, the rubber sheeting was loosened, and the skin at the corners of the gauze squares was marked with a waterproof pen; wrappings and gauze squares were then removed. The test sites were gently washed with warm water to remove excess test substance. The skin was gently patted dry, and the animals were returned to their cages. Approximately 24 and 48 hours after application of the test substance, the test sites were evaluated for erythema, edema, and other evidence of dermal effects, and were scored according to the Draize scale. The adjacent areas of the untreated skin were used for comparison.
GLP:	Yes
Test Substance:	cis-2-Pentenenitrile, purity 98.6%
Results:	No dermal irritation was observed in any of the animals throughout the study. Under conditions of this study, 2-pentenenitrile was not a skin irritant.
Reference:	DuPont Co. (1990). Unpublished Data, Haskell Laboratory Report No. 152-90, "Skin Irritation Test with 2-Pentenenitrile in Rabbits" (April 20) (also cited in TSCA fiche OTS0529919).
Reliability:	High because a scientifically defensible or guideline method was used

Additional Reference for Dermal Irritation:

Data from this additional source were not summarized because insufficient study information was available.

Dow Chemical Co. (1983). R & D Report (March 1) (TSCA fiche OTS0537162).

Type: **Dermal Sensitization:** No Data.

Type:	**Eye Irritation**
Species/Strain:	Female rabbits/New Zealand White
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. On the day of study initiation, the eyes of 2 female rabbits were examined using illumination, magnification, and fluorescein dye. The animals were selected based on no evidence of preexisting corneal or conjunctival injury or irritation. The rabbits were approximately 17 weeks old and weighed 3048 and 2889 g, respectively, on the day of treatment. Approximately 0.01 mL of 2-pentenenitrile was introduced into the lower conjunctival sac of each left eye of 2 rabbits. The right eye was not treated and served as a control. The treated and control eyes of 1 rabbit remained unwashed. Approximately 20 seconds after the test material was administered, both eyes of the remaining rabbit were rinsed for approximately 1 minute with water at room temperature. Each rabbit was observed for approximately 30-60 seconds before being returned to its cage, and any abnormal behavior was noted. Approximately 1 and 4 hours, and 1, 2, and 3 days after the test substance was administered, the rabbits were examined for evidence of eye irritation. At each of these observation periods, eyes were examined using illumination and magnification, and scored for ocular reactions using the Draize scale. Eyes were also observed for any unusual responses to treatment such as pannus, blistering of the conjunctiva, ulceration, or other effects indicative of corrosive action. Reagent strips were used to detect occult blood in discharge from the eye. Biomicroscopic and fluorescein stain examinations were also conducted at post-treatment days 1, 2, and 3. Control eyes were not scored.
GLP:	Yes
Test Substance:	cis-2-Pentenenitrile, purity 98.6%
Results:	2-Pentenenitrile produced moderate conjunctival redness and minimal blood-tinged discharge in the treated washed eye. No irritation was observed in the eye that was washed after treatment. Fluorescein stain was negative for corneal injury, and biomicroscopic examinations revealed no corneal injury

	in both treated eyes throughout the study. The unwashed eye was normal by 2 days after treatment. Under the conditions of this study, 2-pentenenitrile was a moderate eye irritant.
Reference:	DuPont Co. (1990). Unpublished Data, Haskell Laboratory Report No. 158-90, "Eye Irritation Test with 2-Pentenenitrile in Rabbits" (April 11) (also cited in TSCA fiche OTS0529919).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional Reference for Eye Irritation:

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Dow Chemical Co. (1983). R & D Report (March 1) (TSCA fiche OTS0537162).

5.2 Repeated Dose Toxicity

Type:	**28-Day Oral Toxicity**
Species/Strain:	Rats/CRL:CD®(SD)IGS BR
Sex/Number:	Male and female/20 per sex per dose level for the 0 and 75 mg/kg dose groups; 10/sex/dose level for the 10 and 30 mg/kg dose groups
Exposure Period:	All male rats: 28 days (2 month recovery for 10 rats from the 0 and 75 mg/kg dose levels) Female rats designated for subchronic toxicity: 29 days Female rats designated for recovery: 28 days (2 month recovery)
Frequency of Treatment:	Daily
Exposure Levels:	0, 10, 30, 75 mg/kg/day (dosage lowered from 100 mg/kg to 75 mg/kg on test day 8)
Method:	The procedures used in the test were based on the recommendations of the following guideline: OECD Guideline 407. The test substance was administered to male rats for 28 days and female rats for 29 days (females). All groups contained 10 rats/sex/group for evaluation of subchronic toxicity. Control and high-dose groups contained an additional 10 rats/sex/group for evaluation of recovery from test substance-related effects, following a 2-month recovery period.

The high-dose group was reduced on test day 8 from 100 mg/kg to 75 mg/kg, due to mortality and moribundity in rats exposed to 100 mg/kg. For the remainder of the information listed here, this group will be referred to as 75 mg/kg.

Samples containing the test substance at all concentrations were collected on test day 0. These samples were analyzed to determine concentration verification and stability. Samples containing the test substance at all concentrations were collected on test days 14 and 28. These samples were analyzed to determine concentration verification. All dosing solution samples were collected on the same day the solutions were prepared.

Body weights and detailed clinical observations were recorded weekly during the first week of dosing and during the recovery phase, and twice a week during the rest of the dosing phase. In addition, rats designated for neurobehavioral evaluations were weighed on the days of those evaluations. Food consumption was measured weekly.

Ophthalmologic examinations were conducted by a veterinary ophthalmologist. Both eyes were examined by focal illumination and indirect ophthalmoscopy. The examinations were conducted under subdued lighting after mydriasis was produced with a 1% tropic amide solution. On test day –6, the initial examination was performed on all rats received for the study. All surviving rats were examined on test day 22 (near the end of the exposure phase) and on test day 85 (near the end of the recovery phase).

The neurobehavioral groups were given functional observational battery (FOB) assessments (encompassing approximately 37 endpoints) and motor activity (MA) evaluations (encompassing 2 dependent variables) prior to the initiation of exposures, approximately 4 weeks after initiation of dosing, and near the end of the 2-month recovery phase.

Clinical pathology evaluation was conducted on 10 males and females per dose level on the day of necropsy, test day 28 (males) or test day 29 (females). The rats were fasted overnight (approximately 16 hours) and urine was collected over this interval. Each sample was analyzed or examined

for 12 urine parameters.

Blood samples were collected from the same 10 rats/sex/level on the day of necropsy and 14 hematological parameters and 18 clinical chemistry parameters were measured or calculated.

After 28 days (males) or 29 days (females) on study, all surviving rats designated for the subchronic toxicity evaluation were sacrificed and examined for gross and histopathological changes. Rats from the control and high-dose groups designated for recovery were sacrificed after an additional 2 months. The spleen, heart, thymus, liver, kidneys, brain, adrenal glands, testes and epididymides (males), and ovaries and uterus (females) were weighed at necropsy. Each rat was given a gross examination and approximately 50 tissues were saved for microscopic examination. All collected tissues from all animals in the control (0 mg/kg) and high concentration (75 mg/kg) groups were processed and received a full histopathological examination (including prostate, testes, epididymides, seminal vesicles, mammary gland, ovaries, and uterus). Target organs (nose, eyes, liver [females only], spleen, and testes) and most gross lesions were examined from rats in the low (10 mg/kg) and intermediate concentration (30 mg/kg) exposure groups, and from the recovery animals that were sacrificed by design.

Selected neuropathology evaluation was performed on all recovery animals (up to 10/sex/group) in the control and high dose groups, at the end of the recovery period. Based upon in-life evaluations, the sciatic, tibial, and sural nerves were the only neuropathology tissues evaluated.

Body weights, body weight gains, food consumption, food efficiency, and organ weight data were analyzed by one of the 2 following methods: (1) A test for lack of trend was performed. If the preliminary test was not significant a sequential application of the Jonckheere-Terpstra trend test was used. If the preliminary test was significant, preliminary tests for pairwise comparison were used. (2) The Levene's test for homogeneity and Shapiro-Wilk test for normality were performed as a preliminary test. If the preliminary test was not significant, a one-way analysis of variance followed with Dunnett's test was used. If the preliminary test was significant, the Kruskal-Wallis test followed with Dunn's

test was used.

Motor activity was analyzed in a preliminary test using the Levene's test for homogeneity and Shapiro-Wilk test for normality. If the preliminary test was not significant, repeated measures analysis of variance followed by linear contrasts was used. If the preliminary test was significant, sequential application of the Jonckheere-Terpstra trend test was used.

Grip strength and foot splay were analyzed in a preliminary test using Bartlett's test for homogeneity of variances. If the preliminary test was not significant the one-way analysis of variance followed with Dunnett's test was employed. If the preliminary test was significant, the Kruskal-Wallis test followed with Dunn's test was used.

Clinical pathology data was analyzed in a preliminary test using Levene's test for homogeneity and Shapiro-Wilk test for normality. If the preliminary test was not significant, the one-way analysis of variance followed with Dunnett's test was used. If the preliminary test was significant the Kruskal-Wallis test followed with Dunn's test was used.

Incidence of detailed clinical observations and FOB descriptive parameters were analyzed using the Cochran-Armitage test for trend.

GLP: Yes

Test Substance: cis-2-Pentenenitrile, purity 98.77136 (area %)

Results: Analysis of the dosing solutions indicated that the test substance was present at the expected concentration at all dosage levels, and was stable in water under relevant storage conditions.

Survival was reduced in high-dose males. Three males died during the 1st week of the study, when they received 100 mg/kg/day. The deaths were considered compound related. A 4th male from this group died during recovery; the death was considered possibly compound related. No deaths occurred in females.

Compound-related reductions (compared to control) in mean body weight, body weight gain, and food consumption were observed in male and female rats dosed with 10, 30, or 75 mg/kg/day. Mean food efficiency was reduced in male and female rats dosed with 30 or 75 mg/kg/day. Male and female high-dose rats had mean body weight loss over the

first week of dosing, when rats were dosed with 100 mg/kg/day. After the dosage was reduced to 75 mg/kg/day, high-dose rats gained weight, but at a lower rate than control rats. The dose-response for the body weight and nutritional effects demonstrated a similar pattern in males and females, but effects from each dose were generally less severe in females than in males. During recovery, the effects were reversed in both sexes, although mean body weight did not recover completely to control levels. Males demonstrated greater reversal than females.

No compound-related clinical signs of toxicity were observed in rats dosed at 10 or 30 mg/kg. Compound-related clinical signs observed in male and female rats at 100/75 mg/kg included corneal or eye opacity/opaque, head tilt/bob/shake, hyperactivity, hyperreactivity, abnormal gait, and/or circling. The abnormal neurobehavioral signs were mainly observed when the rats were dosed with 100 mg/kg/day, or soon after the dose was reduced to 75 mg/kg/day. None of these neurobehavioral signs was observed during the functional observational battery (FOB) near the end of the dosing phase. The eye opacities observed during detailed clinical observations generally correlated with corneal opacity, keratitis, or uveitis observed during the ophthalmologic examination, with blue haze on the cornea observed during the FOB, and with histopathologic evidence of corneal degeneration. Most of the corneal/eye opacities were resolved after the dose was lowered, prior to the ophthalmologic examination, FOB, and microscopic evaluation. This is reflected in a lower incidence of eye effects observed during these examinations, compared to the incidence during detailed clinical observations.

Neurobehavioral evaluations at the end of the dosing phase demonstrated compound-related effects in the high-dose group, including reductions in forelimb and hindlimb grip strength and motor activity, and increases in the incidence of curled posture or sleeping in the home cage. Reductions in hindlimb grip strength were also observed in rats exposed to 30 mg/kg/day or 10 mg/kg/day (females only). Reduction in hindlimb grip strength was still present in high-dose females at the end of the recovery phase, although the effects were partially reversed. The reductions in grip strength are likely due to the lower body weight gain. No compound-related neuropathology was observed in rats evaluated at the end of the recovery phase.

There were no toxicologically significant effects on coagulation, clinical chemistry, or urinalysis parameters. Compound-related decreases in red cell mass parameters, accompanied by reticulocytosis, were observed in high-dose females at the end of the dosing phase, and were considered adverse. Increased pigment and hematopoiesis were observed in the spleen and were considered to be non-adverse markers for the hematology effects. No adverse clinical pathology effects were observed in recovery female rats or in male rats at either evaluation time.

Compound-related degeneration/atrophy of the dorsal olfactory mucosa, sometimes associated with submucosal fibrosis and degeneration of subjacent olfactory nerve fibers, was observed in all groups dosed with the test substance. Lesion incidence and severity were greatest in the 75 mg/kg/day males and females, slightly less in the 10 mg/kg/day males and females, and least in the 30 mg/kg/day males and females. The explanation for the non-monotonic dose-response for this lesion was not determined. Other toxicology endpoints did not demonstrate a similar pattern and the procedures used in dose solution preparation and dosing supported that all rats received the proper dosage of test substance. The nasal lesions observed at the end of the dosing phase were still present in rats after a 2-month recovery period. Metaplasia of olfactory mucosa to respiratory mucosa, considered to be a reparative process, was also present in recovery rats. Therefore, the nasal effects did not demonstrate reversal. Minimal spermatid retention was observed in testes of males dosed at 75 m/kg. This lesion was considered adverse but was reversible.

Under the conditions of this study, there is no no-observed-effect level (NOEL) for repeated-dose toxicity of the test substance in male or female rats. This lack of a NOEL is based on compound-related reductions in body weight and nutritional parameters, reductions in hindlimb grip strength (females only), and degeneration/atrophy of nasal dorsal olfactory mucosa in male and female rats dosed with 10 mg/kg/day and above. The in-life parameters in high-dose rats were more severe during the week the rats were dosed with 100 mg/kg/day, and demonstrated some recovery when the dosage was reduced to 75 mg/kg/day. Most parameters demonstrated at least partial reversal over the 2-month recovery phase; however, nasal histopathology did not demonstrate reversal.

Reference: DuPont Co. (2001). Unpublished Data, Haskell Laboratory

	Report No. DuPont-5496, "Repeated-Dose Oral Toxicity 28-Day Gavage Study in Rats" (October 23).
Reliability:	High because a scientifically defensible or guideline method was used.
Type:	**4-Week Inhalation Neurotoxicity**
Species/Strain:	Rats/CRL:CD®BR
Sex/Number:	Male and female/15 per sex per dose level
Exposure Period:	28 days
Frequency of Treatment:	6 hours/day, 5 days/week
Exposure Levels:	0, 3, 30, 100, 300 ppm
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

Rats were exposed nose-only to cis-2-pentenenitrile vapor for 6 hours/day over approximately a 4-week period for a total of 20 exposures. Each exposure group was subdivided into 3 replicates of 5 rats. For each group, 1 replicate was designated for evaluation of subchronic toxicity, and the other 2 replicates were designated for neurobehavioral evaluation and neuropathology. One air-exposed control group was subjected to the same treatment and evaluation as the test groups.

During the exposures, rats were individually restrained in perforated, stainless steel or polycarbonate cylinders with conical nose pieces. The restrainers were inserted into the face plate of a 150-L stainless steel exposure chamber so that only the nose of each rat protruded into the chamber. Chamber temperature, relative humidity, and airflow were measured with an automated environmental monitoring system. Chamber oxygen concentrations were targeted to at least 19% and were measured with an oxygen monitor.

Vapors of cis-2-pentenenitrile were dynamically generated by infusing the test substance into a heated 3-neck mixing flask at a particular rate controlled by an infusion pump. Nitrogen, introduced into the heated 3-neck mixing flask, directed the resulting gas into the exposure chamber. Dilution air was delivered to the top of the chamber. The test substance vapor was dispersed using a stainless steel baffle. Atmospheric concentrations were monitored at approximately 45-minute intervals using gas chromatographic analysis.

Rats were weighed and clinical signs of toxicity were recorded. Food consumption was determined for the replicates designated for subchronic toxicity.

Urine samples were collected overnight from all rats/sex/level assigned to the subchronic toxicity group following the 20th exposure for clinical pathological evaluation. Each sample was analyzed or examined for 13 urine parameters.

Blood samples were collected from the same 5 rats/sex/level on the day following the 20th exposure, and 16 hematological parameters and 18 clinical chemistry parameters were measured or calculated. The males were not fasted prior to the evaluation, however the females were fasted overnight after being allowed access to food for approximately 2 hours following removal from the exposure chambers.

The neurobehavioral groups were given functional observational battery (FOB) assessments (encompassing 34 endpoints) and motor activity (MA) evaluations (encompassing 2 dependent variables) prior to the initiation of exposures, and on test days 11, 18, and 32.

At the end of the exposure period all rats assigned to the subchronic toxicity groups (5/sex/level) were sacrificed and examined for gross and histopathological changes. The lungs, liver, kidneys, brain, adrenal glands, and testes were weighed at necropsy. Each rat was given a gross examination and 30 tissues were examined microscopically.

Sacrifices of the neurotoxicity satellite groups were held serially after 1- and 4-weeks recovery (5/sex/group during each sacrifice). All rats had gross pathological and neuropathological examinations performed. Neuropathological assessment included gross examination of the neuromuscular system, and histopathological examination was performed on 15 tissues.

Body weights and body weight gains were analyzed by a one-way analysis of variance. When the corresponding F-test for difference among test group means was significant, pairwise comparisons between test and control groups were made with the Dunnett's test. Incidence of clinical observation was evaluated by the Fisher's Exact test with a

Bonferroni correction and the Cochran-Armitage test for trend.

For clinical pathology data, a one-way analysis of variance (ANOVA) and Bartlett's test were calculated for the sampling time. When the value of F-test statistic from ANOVA was significant, Dunnett's test was used to compare means from the control groups and each of the groups exposed to the test substance. When the results of Bartlett's test were significant ($p \leq 0.005$), the Kruskal-Wallis test was employed and the Mann-Whitney U test was used to compare means from the control groups and each of the groups exposed to the test substance. Significance was judged at the 5% probability level.

Descriptive FOB parameters were evaluated first by Cochran-Armitage test for trend, then by Fisher's Exact test (with a Bonferroni correction) to determine significant experimental group differences with respect to the control group.

Body weights and continuous data from the FOB (fore- and hindlimb grip strength, landing foot splay) were analyzed as parametric data. Bartlett's test of homogeneity of variances was used to estimate the probability that the dose groups had different variances. If Bartlett's test results were not significant, the data were then analyzed via univariate analysis of variance (ANOVA), with Dunnett's test used to identify which dose groups, if any, were significantly different from the control group. If Bartlett's test was significant, data were analyzed via a Kruskal-Wallis test, with the Wilcoxon test used to identify which dose groups, if any, were significantly different from the control group.

Motor activity data were analyzed via univariate analysis of variance with DOSE as a between subjects factor and BLOCK as a repeated measure. In the event of significant effect of DOSE, totals for the control group and groups given the test substance were compared using Dunnett's test. In the event of a significant interaction of DOSE and BLOCK, Dunnett's test was used to identify which dose groups within each block, if any, were significantly different from the control group.

Analysis of grip strength data was conducted on the mean of the absolute scores collected on each of 3 trials conducted at

each test interval (baseline and test days 11, 18, and 23), as well as the mean of the normalized scores collected on the same 3 trials at each interval (calculated as a percent of the baseline grip strength). Similarly, analysis of landing foot splay data was also conducted on both absolute and normalized scores as described previously for the grip strength data.

All significance levels for neurotoxicity parameters were judged at alpha = 0.05, with the exception of Bartlett's test which was judged at alpha = 0.005.

The incidence of gross observations were analyzed by the Fisher Exact test (alpha = 0.05) and the Cochran-Armitage test for trend (alpha = 0.05). The incidences of microscopic findings were analyzed by the Cochran-Armitage test for trend. When a trend was identified, the test was re-run excluding the highest-dose group. This procedure was repeated until either no trend was identified or only the lowest dose group and the control group remained. These 2 incidences were compared using a pair-wise comparison statistic (Fisher Exact test, alpha = 0.05). Final body weights, organ weights, and relative organ weights were statistically evaluated by a one-way analysis of variance (ANOVA). When the test for differences among test group means (value of the F test statistic) was significant (alpha = 0.05), pairwise comparisons between test and control group were made with Dunnett's test (alpha + 0.05). The Bartlett's test for homogeneity of variances (alpha = 0.05) was performed on all weight data in order to justify the use of these parametric parameters.

GLP: Yes

Test Substance: cis-2-Pentenenitrile, purity 73%

Results: The actual mean concentrations of cis-2-pentenenitrile were 0, 3.2, 31, 102, and 292 ppm corresponding to target concentrations of 0, 1, 30, 100, and 300 ppm, respectively.

During exposures, the total airflow for the chambers was targeted at approximately 33 L/min. Chamber temperatures were similar between the 5 chambers, and the mean temperatures ranged from 21-26°C. The relative humidity values were also similar between the chambers and the mean values ranged from 31-47%.

Compound-related mortality did not occur over the course of the exposure period or during the recovery period. The

incidences and type of clinical observations were similar to control for males and females exposed to 3, 30, 100, or 300 ppm. A compound-related decrease in body weight and body weight gain occurred in males at exposure concentrations of 30, 100, and 300 ppm. The lower body weight may be due in part to lower food consumption and lower food efficiency that were observed in males at concentrations of 30 ppm and above, and in females at 300 ppm.

Compound-related hematological alterations were not observed at any exposure concentration in either males or females. Serum sorbitol dehydrogenase activity was decreased in 100 and 300 ppm males and females, and in 30 ppm females. In addition, serum aspartate aminotransferase activity was significantly decreased in the 100 and 300 ppm females. The mechanism(s) related to the decreases in serum enzyme activity have not been determined, however, the decreased activities were considered to be compound-related and potentially adverse. Females exposed to 300 ppm also had increased urine volume and decreased urine osmolality, which were consistent with diuresis. Diuresis was not apparent in males.

There were no compound-related changes in FOB or MA parameters at any exposure concentration in either males or females. There were no compound-related gross or microscopic morphological changes in nervous tissue at any exposure concentration in either males or females.

Differences in absolute and relative organ weights in males were considered to be secondary to decreased body weight. In females, a compound-related increase in absolute and relative liver weights occurred at 300 ppm, and an increase in relative liver weights occurred at 100 ppm, however, microscopic morphological changes were not evident. Compound-related microscopic effects were observed in the nose of both male and female rats exposed to 30 ppm and above. These changes consisted of primary olfactory epithelial degeneration with secondary necrotic exudate, regeneration/metaplasia, and vacuolation of the olfactory nerve bundles.

The no-observable-adverse-effect level (NOAEL) was less than 3 ppm cis-2-pentenenitrile for male rats based on the lower body weights at 3 ppm and above. For females, the

	NOAEL was 3 ppm based on microscopic nasal lesions observed in female rats at 30 ppm and above, and on the changes in sorbitol dehydrogenase activity at 30 ppm and above. The NOAEL for neurotoxicity was 300 ppm in both males and females.
Reference:	DuPont Co. (1993). Unpublished Data, Haskell Laboratory Report No. 170-93, "Four-Week Inhalation Neurotoxicity Study in Rats" (March 31).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Repeated Dose Toxicity:

Data from these additional sources were not summarized because the study design was not adequate.

DuPont Co. (2001). Unpublished Data, Haskell Laboratory, "cis-2-Pentenenitrile" (cited in 8e letter to EPA dated January 8).

DuPont Co. (1995). Unpublished Data, Haskell Laboratory Report No. 655-95, "Range-Finding Neurotoxicity Study in Rats" (November 9) (also cited in TSCA fiche OTS0557945).

Gagnaire, F. and B. Marignac (1999). Pharmacol. Toxicol., 84(6):247-254.

5.3 Developmental Toxicity

Species/Strain:	Rats/Sprague-Dawley
Sex/Number:	*In vitro*: Not specified *In vivo*: Female/3-7
Route of Administration:	*In vitro* *In vivo*: Oral
Exposure Period:	*In vitro*: 46 hours *In vivo*: 1 day
Frequency of Treatment:	Once
Exposure Levels:	*In vitro*: 0, 1.75, 2.5, 5, 7.5, 10 mM, and 1.75 + microsomes + NADPH *In vivo*: 125 mg/kg
Method:	No specific test guideline was reported. *In vitro experiment:* Nulliparous female Sprague-Dawley rats were caged with adult males for a 2-hour period. Successful mating was ascertained by the presence of sperm in the vaginal smear, and the following 24 hours was termed Day 0 of gestation (GD). Conceptuses were explanted from

uteri of 10-day pregnant dams and were dissected free of decidua and Reichert's membrane leaving the visceral yolk sac, amnion, and ectoplacental cone intact. Embryos selected for culture were at neural plate stage and ventrally convex. Two conceptuses were placed in a culture bottle containing inactivated pure male rat serum. The cultures were grown in the absence of antibiotics.

The test substance was dissolved in dimethyl sulfoxide (DMSO) and was added to the culture medium with or without a P-450-dependent hepatic bioactivating system. A concurrent vehicle control was used. Explants were cultured for 46 hours with a constant rotation at 37°C. Culture bottles were initially gassed with a mixture of O_2:CO_2:N_2 (5:5:90) and were regassed with a mixture of O_2:CO_2: N_2 (20:5:75) after 20 hours, O_2:CO_2:N_2 (40:5:55) after 28 hours, and O_2:CO_2 (95:5) after 44 hours of culture. Conceptuses were removed from the culture bottles after 46 hours. Only viable embryos (embryos with a beating heart) were examined for growth and morphological development. The yolk sac was assessed for its vascularization and circulation, and its diameter was recorded. The crown-rump length and head length were measured and the number of somite pairs was noted. Crown-rump length was not recorded in embryos with defective flexion. Morphological features including embryonic flexion, heart, neural tube, brain development, otic, optic, and olfactory systems, branchial bars, maxillary and mandibular processes, and limb buds were examined.

In vivo experiment: Pregnant Sprague-Dawley rats were given a single oral administration of 125 mg/kg cis-2-pentenenitrile, dissolved in olive oil, on GD10. The pregnant dams were euthanized on GD12 and the numbers of implantation sites and of embryos with a beating heart were recorded.

GLP: Unknown

Test Substance: cis-2-Pentenenitrile, purity > 98%

Results: *In vitro experiment:* The development of embryos exposed to 1.75 and 2.5 mM cis-2-pentenenitrile was similar to that of controls. Embryos exposed to 5.0 mM and above showed significant decreases in all 4 measurements of conceptus growth (yolk sac diameter, crown-rump length, head length, and number of somites), and developmental, as well as morphological alterations. The principal sites affected were the prosencephalon, the mesencephalon, and the maxillary processes, and, to a lesser extent, the auditory system. At

10 mM, 28% of the embryos were dead, and all the surviving embryos were malformed.

Inclusion of microsomes and NADPH markedly enhanced the embryotoxic effects of 1.75 mM cis-2-pentenenitrile. The incidence of embryos with morphological defects rose from 0 to 67%. Multiple anomalies were found in about 1/3 of the embryos. Growth reduction also appeared. The yolk sac diameter, crown-rump length, head length, and number of somites of embryos exposed to 1.75 mM cis-2-pentenentrile with microsomes were significantly decreased compared to control or to 1.75 mM cis-2-pentenenitrile without microsomes (9-23% lower). In addition to the morphological defects already observed in embryos exposed to 5-10 mM cis-2-pentenenitrile alone, 1.75 mM cis-2-pentenenitrile plus the biotransformation system elicited changes in the somites 3-8 region in 56% of the embryos.

In vivo experiment: Clinical signs of toxicity were observed in treated dams (e.g., piloerection, prostration, and/or tremors). Cis-2-pentenenitrile caused maternal weight loss between GD10 and GD12. Embryo viability was not affected 48 hours after maternal dosing with cis-2-pentenenitrile on GD10. Up to 94% of the embryos (4 litters examined) exhibited allantois, trunk, and caudal extremity misdirected.

Reference: Saillenfait, A. M. and J. P. Sabate (2000). Toxicol. Appl. Pharm., 163(2):149-163.

Reliability: Low because an inappropriate method or study design was used.

Additional References for Developmental Toxicity: None Found.

5.4 Reproductive Toxicity: No Data.

5.5 Genetic Toxicity

Type:	***In vitro* Bacterial Reverse Mutation Test**
Tester Strain:	*Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537
Exogenous Metabolic Activation:	With and without 10% and 30% Aroclor®-induced rat and hamster liver S-9
Exposure Concentrations:	Initial Trial: 0, 33, 100, 333, 1000, 2000, and 3333 µg/plate Subsequent Trials: 0, 100, 333 or 334, 667, 1000, 2000,

3333 or 3334, 5000, and 6667 μg/plate
Comment: Not all exposure concentrations were tested with all tester strains under all test conditions.

Method: No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

The preincubation method originally described by Haworth et al., 1983, was used with some modifications. The test substance, overnight culture of *Salmonella*, and S-9 mix or buffer were incubated at 37°C, without shaking for 20 minutes. Test substances known or suspected to be volatile were incubated in capped tubes. The top agar was added and the contents of the tubes were mixed and poured onto the surface of petri dishes containing medium. Histidine-independent (his+) colonies arising on these plates were counted following 2 days incubation at 37°C. Plates were machine counted (New Brunswick, Artek). At the discretion of the investigator, plates with low numbers of colonies, containing precipitated test substance, or having excessively-reduced contrast because of chemical color, were counted by hand.

The initial test of a test substance was without activation and with 10% S-9. If a positive result was obtained, the positive trial(s) was repeated. If the trials were negative, the test substance was retested without S-9 and with 30% S-9. If all trials were negative, no further testing was performed.

A test substance was designated nonmutagenic only after it had been tested in strains TA97, TA98, TA100, TA1535, and TA1537, without exogenous activation, and with 10% and 30% rat and hamster S-9.

2-Pentenenitrile was run initially in a toxicity assay using TA100 or the system developed by Waleh et al., 1982. Toxic concentrations were defined as those that produced a decrease in the number of his+ colonies, or a clearing in the density of the background lawn, or both.

The test substance was initially tested in the preincubation test at half-log dose intervals up to a dose that elicited toxicity, or to a dose immediately below one that was toxic in the preliminary toxicity procedure. Subsequent trials occasionally used narrower dose increments, and may not have included doses in the toxic range. At least 5 doses of

	the test substance were tested in triplicate, and repeat experiments were performed at least 1 week following the initial trial. Concurrent solvent (dimethyl sulfoxide) and positive controls were run with each trial. The positive controls in the absence of exogenous metabolic activation were sodium azide (TA1535 and TA100), 9-aminoacridine (TA97 and TA1537), and 4-nitro-o-phenylenediamine (TA98). The positive control for exogenous metabolic activation with all strains was 2-aminoanthracene. The test substance was considered mutagenic or weakly mutagenic if it produced a reproducible, dose-related response over the solvent control, under a single metabolic activation condition, in replicate trials. The test substance was considered equivocal if the results of individual trials were not reproducible, if increases in his+ revertants did not meet the criteria for a weakly positive response, or if only single doses produced increases in his+ revertants in repeat trials. The test substance was judged nonmutagenic if it did not meet the criteria for a mutagenic or questionable response.
GLP:	Unknown
Test Substance:	2-Pentenenitrile, purity 70%
Results:	Negative
Remarks:	2-Pentenenitrile was cytotoxic as indicated by a slight clearing of the background lawn and a reduction of revertants at concentrations starting at 3333 μg/plate in the presence of metabolic activation. In the non-activated condition, indications of toxicity was observed with some strains, but the magnitude seemed to be less pronounced. 2-Pentenenitrile was not mutagenic, with or without exogenous activation in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537.
Reference:	Zeiger, E. et al. (1992). Environ. Mol. Mutagen., 19(Suppl. 21):2-141. Haworth, S. et al. (1983). Environ. Mutagen., 6(Suppl. 1):3-142. Waleh, N. S. et al. (1982). Mutat. Res., 97:247-256
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for *In vitro* Bacterial Reverse Mutation Studies:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

DuPont Co. (1978). Unpublished Data, Haskell Laboratory Report No. 752-78, "Mutagenic Activity in the *Salmonella*/Microsome Assay" (January 16) (also cited in TSCA fiche OTS0529919).

DuPont Co. (1990). Unpublished Data, Haskell Laboratory Report No. 621-90, "Mutagenicity Testing of cis-2-Pentenenitrile in the *Salmonella typhimurium* Plate Incorporation Assay" (January 25).

DuPont Co. (1990). Unpublished Data, Haskell Laboratory Report No. 425-90, "Mutagenicity Testing of 2-Pentenenitrile in the *Salmonella typhimurium* Plate Incorporation Assay" (September 13) (also cited in TSCA fiche OTS0529919).

Type:	***In vitro* Mouse Lymphoma Mutation Assay**
Cell Type:	Mouse lymphoma cells (L5178Y; TK locus)
Exogenous Metabolic Activation:	With and without Aroclor®-induced rat liver S-9
Exposure Concentrations:	Nonactivated cultures: 0.1 to 1.0 μg/mL Activated cultures: 0.0040 to 0.04 μg/mL
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. The initial toxicity test conducted on the test substance indicated threshold levels of complete toxicity at 1.0 μg/mL for the nonactivated cultures, and at 0.5 μg/mL for the S-9 activated cultures. The test substance was tested in the mutagenesis assay over a range of concentrations from 0.10 to 1.0 μg/mL for the nonactivated cultures, and from 0.004 to 0.040 μg/mL for the S-9 activated cultures. Duplicate cultures were treated. After a 2-day expression period, 12 nonactivated cultures and 5 S-9 activated cultures were selected for cloning based on their degree of toxicity. The nonactivated cultures were cloned at test substance concentrations of 0.87, 0.74, 0.61, 0.49, 0.36, and 0.23 μg/mL. The S-9 activated cultures were cloned at test substance concentrations of 0.014, 0.0091, and

	0.0040 μg/mL.
GLP:	Yes
Test Substance:	cis-2-Pentenenitrile, purity not specified
Results:	Positive
Remarks:	Both the nonactivated and S-9 activated cultures produced significant increases in mutant frequency over that of the solvent control cultures. For cultures treated in the absence of S-9 metabolic activation, 4 of the cultures exhibited an increase in mutant frequency, which ranged from 3.3 to 6.3 times greater than the average mutant frequency of the solvent control cultures. The total growth of these cultures ranged from 9-29%, and a dose-dependent response was evident. The remaining cultures did not exhibit significant increases in mutant frequency. The total growth of these cultures ranged from 44-98%. For cultures treated in the presence of exogenous S-9 metabolic activation, 3 of the cultures exhibited significant increases in mutant frequency, which ranged from 2.0 to 4.2 times greater than the average mutant frequency of the corresponding solvent control cultures. The total growth of these cultures ranged from 5-25%. Neither of the remaining cultures exhibited a significant increase in mutant frequency. The total growth of these cultures were at 74% and 61%.
Reference:	National Cancer Institute (1983). Microbiological Associates Study No. ML-NCI #70, Contract No. N01-CP-15739.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for *In vitro* Studies: None Found.

Type: ***In vitro* Clastogenicity Studies:** No Data.

Type: ***In vivo* Studies:** No Data.

Appendix C

ROBUST SUMMARY FOR 3-PENTENENITRILE

Existing published and unpublished data were collected and scientifically evaluated to determine the best possible study or studies to be summarized for each required endpoint. In the spirit of this voluntary program, other data of equal or lesser quality are not summarized, but are listed as related references at the end of each appropriate section, with a statement to reflect the reason why these studies were not summarized.

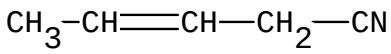
1.0 Substance Information

CAS Number: 4635-87-4 (3-pentenenitrile)
16545-78-1 (cis-3-pentenenitrile)
16529-66-1 (trans-3-pentenenitrile)

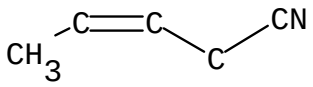
Chemical Name: 3-Pentenenitrile

Structural Formula:

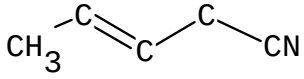
3-Pentenenitrile



cis-3-Pentenenitrile



trans-3-Pentenenitrile



Other Names: <u>3-Pentenenitrile</u>

3-PN
3PN
1-Cyano-2-butene
3-Pentenonitrile
Crotyl cyanide

<u>trans-3-Pentenenitrile</u>

3-Pentenenitrile, (3E)-
(E)-3-Pentenenitrile
(E)-2-Butenyl cyanide
trans-1-Cyanobut-2-ene
trans-2-Butenyl cyanide
trans-3-Pentenenitrile
trans-3-Pentenonitrile

<u>cis-3-Pentenenitrile</u>

3-Pentenenitrile, (3Z)-
3-Pentenenitrile, (Z)-

Exposure Limits: 1 ppm; 8- and 12-hour TWA; skin notation: DuPont Acceptable Exposure Limit (AEL) (3-pentenenitrile)

2.0 Physical/Chemical Properties

2.1 Melting Point

Value:	-46.66
Decomposition:	No Data
Sublimation:	No Data
Pressure:	760 mm Hg
Method:	Modeled. MPBPWIN, v.1.4, module of EPIWIN 3.05 (Syracuse Research Corporation). MPBPWIN estimates melting point by two different methods. The first is an adaptation of the Joback group contribution method for melting point (Joback, 1982; Reid et al., 1987) and the second is a simple Gold and Ogle method suggested by Lyman, 1985.
GLP:	Not Applicable
Reference:	Joback, K. G. (1982). A Unified Approach to Physical Property Estimation Using Multivariate Statistical Techniques. Stevens Institute of Technology, submitted to the Dept. of Chem. Eng. For M.S. Degree at the Massachusetts Institute of Technology in June 1984 (see also: Reid et al., 1987). Reid, R. C. et al. (1987). The Properties of Gases and Liquids, 4th edition, Chapter 2, Mc-Graw-Hill, Inc., NY. Lyman, W. J. (1985). In: Environmental Exposure From Chemicals, Volume I, Chapter 2, Neely, W. B. and G. E. Blau (eds.), CRC Press, Inc., Boca Raton, FL.
Reliability:	Estimated value based on accepted model.

SUPPORTING DATA (1-pentenenitrile)

Value:	-96°C
Decomposition:	No Data
Sublimation:	No Data
Pressure:	No Data
Method:	No Data
GLP:	No Data
Reference:	Lievens (1924). Bull. Soc. Chim. Belg., 22:127 (Beilstein Database, accessed June 17, 2003).

Timmermans (1927). Bull. Soc. Chim. Belg., 36:507 (Beilstein Database, accessed June 17, 2003).

Timmermans and Delcourt (1934). J. Chim. Phys. Phys. Chim. Biol., 31:110 (Beilstein Database, accessed June 17, 2003).

Joutkovsky (1934). Bull. Soc. Chim. Belg., 43:401 (Beilstein Database, accessed June 17, 2003).

Witschonke (1954). Anal. Chem., 26:562 (Beilstein Database, accessed June 17, 2003

Reliability: Not assignable because limited study information was available.

SUPPORTING DATA (9-octadecenenitrile)

Value:	-1°C
Decomposition:	No Data
Sublimation:	No Data
Pressure:	No Data
Method:	No Data
GLP:	Unknown
Reference:	Weast, R.C. (ed.) (1979). Handbook of Chemistry and Physics, 60th ed., p. C-404, CRC Press Inc., Boca Raton, Florida.
Reliability:	Not assignable because limited study information was available.

Additional References for Melting Point: None Found.

2.2 Boiling Point

Value:	144-147ºC
Decomposition:	Decomposes with heat
Pressure:	760 mm Hg
Method:	No Data
GLP:	Unknown
Reference:	DuPont Co. (1998). Material Safety Data Sheet DU005951 (September 18).
Reliability:	Not assignable because limited study information was available.

Additional Reference for Boiling Point:

DuPont Co. (n.d.) Data Sheet, "3-Pentenenitrile."

2.3 Density

Value:	0.83
Temperature:	20ºC
Method:	No Data
GLP:	Unknown
Results:	No additional data.
Reference:	DuPont Co. (1998). Material Safety Data Sheet DU005951 (September 18).
Reliability:	Not assignable because limited study information was available.

Additional References for Density: None Found.

2.4 Vapor Pressure

Value:	4.05 mm Hg
Temperature:	25°C
Decomposition:	No Data
Method:	Estimated using the means of Antoine & Grain methods.
GLP:	Not Applicable
Reference:	SRC MPBPWIN v1.40 in EPIWIN v3.05.
	Syracuse Research Corporation (MPBPWIN) program estimates the boiling point (at 760 mm Hg), melting point, and vapor pressure of organic compounds. The vapor pressure is estimated using the mean of the Antoine and Grain methods. A description of the methodology is detailed in:
	Antoine Method: Lyman, W. J. et al. (1990). Handbook of Chemical Property Estimation Methods, Chapter 14, American Chemical Society, Washington, DC.
	Modified Grain Method: Lyman, W. J. (1985). In: Environmental Exposure From Chemicals, Volume I, Chapter 2, Neely, W. B. and G. E. Blau (eds.), CRC Press, Inc., Boca Raton, FL.
Reliability:	Estimated value based on accepted model.

Additional Reference for Vapor Pressure:

DuPont Co. (1998). Material Safety Data Sheet DU005951 (September 18).

2.5 Partition Coefficient (log Kow)

Value:	1.11
Temperature:	Not Applicable
Method:	Modeled. The KOWWIN computer program, version 1.66 from Syracuse Research Corporation, calculates the Log octanol/water partition coefficient (log Kow) of organic chemicals using an atom/fragment contribution method.
GLP:	Not Applicable
Reference:	The methodology is described in the following journal article: Meylan, W. M. and P. H. Howard (1995). J. Pharm. Sci., 84:83-92.
Reliability:	Estimated value based on accepted model

Additional References for Partition Coefficient (log Kow): None Found.

2.6 Water Solubility:

Value:	7924 mg/L
Temperature:	22.5°C
pH/pKa:	No Data
Method:	Water solubility of the test substance was estimated by determining the total organic carbon in water to which an amount of test substance in excess of the water solubility had been added. Samples were tested for their solubility in water after 48 and 96 hours of continuous mixing. Approximately 200 mg of the test substance, prepared in quadruplicate replicates, was added to 20 mL of deionized water in a glass test vessel with a Teflon®-coated screw cap. Two of the replicates were mixed end-over-end for 48 hours and then analyzed. The other 2 replicates were mixed end-over-end for 96 hours before analysis. Prior to analysis, the test vessels were allowed to stand for 1 hour. The upper 5 mL layer of solution was removed by pipette and discarded to eliminate test substance at the top of the test vessel. Samples were analyzed for total carbon content via an analyzer with an autosampler attachment. Water solubility was determined by assuming that the test material contained 100% test substances.
GLP:	No
Reference:	DuPont Co. (2002). Unpublished Data, Report No. EMSER 006-02, "Estimated Water Solubility of 3-Pentenenitrile" (January 24).

Reliability:	Medium because a suboptimal study design was used.
Value:	7930 mg/L
Temperature:	25°C
pH/pKa:	No Data
Method:	Modeled
GLP:	Not Applicable
Reference:	WsKow v1.40 in EPIWIN v3.05 (SRC Database). WsKow estimates the water solubility (Wsol) of an organic compound using the compound's log octanol-water partition coefficient (log Kow). The following journal article describes the estimation methodology: Meylan, W. M. et al. (1996). Environ. Toxicol. Chem., 15:100-106.
Reliability:	Estimated value based on accepted model.

Additional References for Water Solubility: None Found.

2.7 Flash Point

Value:	40ºC
Method:	Closed cup
GLP:	Unknown
Reference:	DuPont Co. (1998). Material Safety Data Sheet DU005951 (September 18).
Reliability:	Not assignable because limited study information was available.

Additional Reference for Flash Point:

DuPont Co. (n.d.). Data Sheet, "3-Pentenenitrile."

2.8 Flammability

Results:	Vapor forms explosive mixture with air.
Method:	No Data
GLP:	Unknown
Reference:	DuPont Co. (1998). Material Safety Data Sheet DU005951 (September 18).
Reliability:	Not assignable because limited study information was available.

Additional References for Flammability: None Found.

3.0 Environmental Fate

3.1 Photodegradation

Concentration:	Not Applicable
Temperature:	Not Applicable
Direct Photolysis:	Using the absorption spectrum of acetonitrile as an analog example, the nitrile group does not absorp significantly above 200 nm: absorbance at 200 nm = 0.04 absorbance at 210 nm = 0.03 absorbance at 220 nm = 0.01 absorbance at 254 nm = 0.005. Harris (1990) also reported that ethylene, an analog for C=C in the unsaturated mononitriles, has no significant absorption above 290 nm. Therefore, the mononitrile category is expected to lack significant absorptivity above 290 nm and will not be subject to direct photolysis.
Indirect Photolysis:	In the vapor phase, cis 3-pentenenitrile is estimated to have an atmospheric half-life of 13.5 hours due to hydroxyl radical oxidation and a half-life of 2.1 hours due to reactions with ozone. The two reactions result in an estimated atmospheric half-life of 1.82 hours for vapor phase material. In the vapor phase, trans 3-pentenenitrile is estimated to have an atmospheric half-life of 11.9 hours due to hydroxyl radical oxidation and a half-life of 1.4 hours due to reactions with ozone. The two reactions result in an estimated atmospheric half-life of 1.25 hours for vapor phase material.
Breakdown Products:	Not Applicable
Method:	The AOP Program, Version 1.90 from Syracuse Research Corporation, estimates the Atmospheric Oxidation Potential. The AOP program estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The methodology used by the Atmospheric Oxidation Program is based upon the structure-activity relationship (SAR) methods developed by Dr. Roger Atkinson and co-workers (Atkinson et al., 1987; 1995; 1996; 1984). The rate constant for the reaction of 3-pentenenitrile vapor with photochemically generated hydroxyl radicals in the atmosphere is estimated to be 2.8×10^{-11} cm^3/molecule-sec for the cis-isomer and 3.2×10^{-11} cm^3/molecule-sec for the trans

isomer at 25°C (SRC AopWin v1.90). These values correspond to respective half-lives of 0.6 days and 0.5 days, assuming a 24 hour day and an ambient hydroxyl radical concentration of $0.5x10^6$ molecules/cm^3.

GLP: Not Applicable

Reference: Atkinson, R. et al. (1987). Intern. J. Chem. Kinet., 19 :799-828.

Atkinson, R. et al. (1995). Atmos. Environ., 29:1685-1695.

Atkinson, R. et al. (1996). Environ. Sci. Technol., 30:329-334.

Atkinson, R. et al. (1984). Chem. Rev., 84:437-470.

Harris, J. C. (1990). Rate of Aqueous Photolysis, Chapter 8, In: Lyman, W. J. et al. (eds.). Handbook of Chemical Property Estimation Methods, American Chemical Society, Washington, DC.

The following journal article describes the AOP Program:

Meylan, W. M. and P. H. Howard (1993). Chemosphere, 26:2293-2299.

Reliability: Estimated value based on accepted model.

Additional References for Photodegradation: None Found.

3.2 Stability in Water

Concentration: Not Applicable

Half-life: The Henry's Law constant for 3-pentenenitrile is estimated to be $6.29x10^{-5}$ atm-m^3/mole (SRC HENRYWIN v3.10 in EPIWIN v3.05) from its estimated vapor pressure of 4.05 mm Hg (SRC MPBPWIN v1.40 in EPIWIN v3.05, mean of Antoine & Grain methods) and water solubility of 7930 mg/L (WsKow v1.40 in EPIWIN v 3.05). The estimated volatilization half-life from a model river (1 m deep, flowing 1 m/sec, wind velocity of 5 m/sec) is approximately 9.3 hours. The estimated volatilization half-life from a model lake (1 m deep, flowing 0.05 m/sec, wind velocity of 0.5 m/sec) is approximately 7.4 days (EPIWIN v3.05).

% Hydrolyzed: Not Applicable

Method: Modeled. The WVOL program estimates the volatilization half-lives from a model river and lake using the

methodology from Lyman et al., 1990 (adsorption to suspended solids and sediments is ignored). The user can input an experimental water solubility, vapor pressure, or Henry's Law constant or EPI will automatically estimate a Henry's Law Constant from SRC's Henry program for this calculation. WsKow estimates the water solubility (Wsol) of an organic compound using the compound's log octanol-water partition coefficient (Kow).

GLP: Not Applicable

Reference: Lyman, W. J. et al. (1990). The Handbook of Chemical Property Estimation Methods, American Chemical Society.

The following journal article describes the estimation methodology:

Meylan, W. M. et al. (1996). Environ. Toxicol. Chem., 15:100-106.

Reliability: Estimated value based on accepted model.

Additional References for Stability in Water: None Found.

3.3 Transport (Fugacity)

Media: Air, Water, Soil, and Sediments

Distributions:

Air:	0.5%
Water:	45.1 %
Soil:	54.3%
Sediments:	0.1%

Half-life:

Air:	1.8 hours
Water:	360 hours
Soil:	720 hours
Sediment:	3240 hours

Adsorption Coefficient: Not Applicable

Desorption: Not Applicable

Volatility: Not Applicable

Method: Calculated according to Mackay, Level III, Syracuse Research Corporation EPIWIN version 3.05. Emissions (1000 kg/hr) to air, water, and soil compartments using standard EPA model defaults with BIOWIN half-life factors of water, 1; soil, 2; and sediments, 9.

Data Used:
Molecular Weight: 81.12
Chemical Name: 3-Pentenenitrile
Henry's Law Constant: 6.29×10^{-5} atm-m^3/mole (HenryWin

	Program) Vapor Pressure: 4.05 mm Hg (MPBPWIN v1.40) Log Kow: 1.11 (KowWin Program) Soil Koc: 5.28 (Log Kow estimate)
GLP:	Not Applicable
Reference:	Syracuse Research Corporation EPIWIN v3.05 contains a Level III fugacity model. The methodology and programming approach were developed by Dr. Donald MacKay and coworkers and are detailed in: Mackay, D. (1991). Multimedia Environmental Models: The Fugacity Approach, pp. 67-183, Lewis Publishers, CRC Press. Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1618-1626. Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1627-1637
Reliability:	Estimated value based on accepted model.

Additional References for Transport (Fugacity): None Found.

3.4 Biodegradation:

Value:	21% after 28 days (Not Readily Biodegradable)
Breakdown Products:	No Data
Method:	The procedures used in the test were based on the recommendations of the following guideline: OECD Guideline 301B. The biodegradability of 3-pentenenitrile was tested using the Modified Sturm test. Biodegradability was measured as CO_2 evolution. A test substance is considered "Readily Biodegradable" if it demonstrates a "pass level" of 60% biodegradability within a 10-day window after exceeding the 10% level of biodegradability. A test substance is considered "Ultimately Biodegradable" if it demonstrates a "pass level" of 60% biodegradability, but not within a 10-day window after exceeding the 10% level of biodegradability. 3-Pentenenitrile reached a peak of 21% biodegradability at day 28, and therefore is regarded as not "Readily

	Biodegradable." 3-Pentenenitrile was not inhibitory to microorganisms in the inoculum.
GLP:	No
Reference:	DuPont Co. (2001). Unpublished Data, Report No. EMSE-071-01, "Biodegradability of 3-Pentenenitrile Using the Modified Sturm Test (OECD 301B)" (December 17).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Biodegradation: None Found.

3.5 Bioconcentration

Value:	BCF = 1.44. This BCF value suggests that bioconcentration potential in aquatic organisms is low.
Method:	The bioconcentration factor is calculated by Syracuse Research Corporation's BCFWIN Computer Program, Version 2.14, which utilizes a linear regression based on the Log Kow for the compound.
GLP:	Not Applicable
Reference:	The estimation methodology used by BCFWIN is described in the following document prepared for the U.S. Environmental Protection Agency (OPPT): "Improved Method for Estimating Bioconcentration Factor (BCF) from Octanol-Water Partition Coefficient," SRC TR-97-006 (2^{nd} Update), July 22, 1997; prepared for Robert S. Boethling, EPA-OPPT, Washington, DC, Contract No. 68-D5-0012; prepared by William M. Meylan, Philip H. Howard, Dallas Aronson, Heather Printup, and Sybil Gouchie, Syracuse Research Corp., Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212.
Reliability:	Estimated value based on accepted model.

Additional References for Bioconcentration: None Found.

4.0 Ecotoxicity

4.1 Acute Toxicity to Fish

Type:	**96-hour LC_{50}**
Species:	*Pimephales promelas* (fathead minnow)
Value:	>100 mg/L
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used that was consistent with OECD Guideline 203, with the following exceptions: 10x dose spacing, 4 test concentrations, and

nominal test concentrations were reported.

The acute toxicity to fathead minnows was determined in an unaerated, 96-hour, static test. The nominal concentrations of 3-pentenenitrile used were 0, 0.10, 1, 10, and 100 mg/L at a mean temperature of 21.6°C. One test chamber was used per test concentration with 10 test organisms in each chamber.

Analysis of the test and control solution samples for dissolved oxygen and pH were made at test initiation (0 hours) and test completion (96 hours).

GLP: No

Test Substance: 3-Pentenenitrile, purity 98%

Results: Based on visual observations, the water control and the 0.1, 1, 10, and 100 mg/L test concentrations were clear and colorless at test start. All water quality parameters were within acceptable limits during the exposure. At test initiation (0 hours), dissolved oxygen was 8.7 mg/L and pH ranged from 7.5-7.6. At test completion (96 hours) dissolved oxygen ranged from 6.0-6.8 and pH was 7.5.

Exposure of fathead minnows to nominal concentrations of 0, 0.1, 1, 10, and 100 mg/L 3-pentenenitrile resulted in 0% mortality at any concentration at the end of 96 hours. The test substance exhibited low concern for aquatic hazard in the unaerated, 96-hour, static, acute test using the fathead minnow.

Reference: DuPont Co. (2001). Unpublished Data, Haskell Laboratory Report No. DuPont-8175, "Static, Acute, 96-Hour Screening Test to *Pimephales promelas*" (November 19).

Reliability: Medium because a suboptimal study design was used (nominal test concentrations).

Type: **96-hour LC_{50}**

Species: Fathead minnow

Value: 474 µg/L; log Kow = 1.11

Method: Modeled

GLP: Not Applicable

Test Substance: 3-Pentenenitrile

Results: No additional data.

Reference: Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by

Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).

Reliability: Estimated value based on accepted model.

SUPPORTING DATA: 3-BUTENENITRILE

Type: **96-hour LC_{50}**
Species: *Pimephales promelas* (fathead minnow)
Value: 182 mg/L (confidence limits, 171-195 mg/L)
Method: Gas-liquid chromatography (GLC) was used to analyze toxicants in water samples from the fish exposure tanks. All test chambers were sampled at approximately mid-depth at 0, 24, 48, 72, and 96 hours in all exposure chambers. All samples were analyzed immediately or adequately preserved for later analysis.

Five water quality parameters (temperature, dissolved oxygen, total hardness, total alkalinity, and pH) were routinely measured for each test. Temperature measurements were made in each exposure chamber daily. Dissolved oxygen was determined in the high, medium, low, and control exposure chambers at least 3 times (0, 24, and 96 hours) during a test if surviving fish existed in that chamber. Total hardness and total alkalinity were determined at least once for each test. pH was measured at least once during each test in high, medium, low, and control exposure chambers.

Fathead minnows (approximately 33 days old) were exposed to nominal concentrations of 0, 73.3, 113, 173, 267, and 410 mg/L. Fish were not fed during chemical exposure. During the exposure, fish were routinely observed for behavioral responses (effects) and deaths. Death was defined as the cessation of opercular movements and the inability to respond when prodded. Dead fish were removed and recorded at 3, 6, 12, 24, 48, 72, and 96 hours from initial exposure. At the termination of tests, control fish were weighed (wet) to the nearest mg after blotting excess water from them with a paper towel and measured (standard length) to the nearest mm.

Exposure of fish was done in flow-through exposures, with a modified continuously proportioning diluter without duplicate exposures. The modification was the elimination of flow booster and self-siphoning flow splitting cells. Diluters were calibrated. The test substance was

	proportionally diluted with Lake Superior water from stock solutions before delivery to fish exposure chambers.
GLP:	Unknown
Test Substance:	3-Butenenitrile, purity 98.6%
Results:	Average measured test concentrations were 73.8, 105, 166, 240, and 350 mg/L for the 0, 73.3, 113, 173, 267, and 410 mg/L nominal concentrations. Measured water quality parameters included temperature of 25°C, dissolved oxygen of 5.9 mg/L, hardness of 46.0 mg/L $CaCO_3$, alkalinity of 42.0 mg/L $CaCO_3$, and pH of 7.70. Mortality at 96 hours was 0/20, 0/20, 0/20, 3/20, 20/20, and 20/20 at 0, 73.8, 105, 166, 240, and 350 mg/L. Affected fish had abdominal swelling and lost equilibrium prior to death.
Reference:	Brooke, L. T. et al. (eds.) (1984). Acute Toxicities of Organic Chemicals to Fathead Minnows (*Pimephales promelas*), Center for Lake Superior Environmental Studies, University of Wisconsin-Superior, Superior, Wisconsin.
Reliability:	High because a scientifically defensible or guideline method was used.

Type:	**96-hour LC_{50}**
Species:	Fathead minnow
Value:	447 µg/L; log Kow = 0.7
Method:	Modeled
GLP:	Not Applicable
Test Substance:	3-Butenenitrile
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Fish: None Found.

4.2 Acute Toxicity to Invertebrates

Type:	**48-hour EC_{50}**
Species:	*Daphnia magna*
Value:	>100 mg/L
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used that was

consistent with OECD Guideline 202, with the following exceptions: 10x dose spacing, 4 test concentrations, nominal test concentrations were reported, and 1 replicate per test concentration was performed.

The acute toxicity of 3-pentenenitrile to the water flea, *Daphnia magna* (less than 24-hours old) was determined in an unaerated, 48-hour, static test. The study was conducted at concentrations of 0, 0.1, 1.0, 10, and 100 mg/L at a mean temperature of 20.2°C (range of 19.8-20.5°C). One test chamber was used per test concentration with 10 test organisms in each chamber.

GLP: No

Test Substance: 3-Pentenenitrile, purity 98%

Results: Based on visual observations, the water control and the 0.1, 1, 10, and 100 mg/L test concentrations were clear and colorless at test start. All water quality parameters were within acceptable limits during the exposure. Dissolved oxygen was 8.7-8.8 and 8.5-8.6 mg/L at test initiation (0 hours) and test completion (48 hours), respectively. pH was 7.4-7.8 and 7.8-7.9 at test initiation (0 hours) and test completion (48 hours), respectively.

Immobility was 0% in all test concentrations at the end of 48 hours. No immobility or sublethal effects were observed in the water control test organisms. The highest nominal concentration causing no immobility at test end was 100 mg/L. The test substance exhibited low concern for aquatic hazard in an unaerated, 48-hour, static, acute test using *Daphnia magna* (less than 24 hours old).

Reference: DuPont Co. (2001). Unpublished Data, Haskell Laboratory Report No. DuPont-8176, "Static, Acute, 48-Hour Screening Test to *Daphnia magna*" (October 26).

Reliability: Medium because a suboptimal study design was used (nominal test concentrations).

Additional References for Acute Toxicity to Invertebrates: None Found.

4.3 Acute Toxicity to Aquatic Plants: No Data.

5.0 Mammalian Toxicity

5.1 Acute Toxicity

Type:	**Oral ALD**
Species/Strain:	Male rats/ChR-CD
Value:	300 mg/kg
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. 3-Pentenenitrile, as a solution in peanut oil, was administered in single doses via intragastric intubation to young adult male rats at 90, 130, 200, 300, 450, 670, 2250, or 5000 mg/kg. Survivors were sacrificed 14 days later.
GLP:	No
Test Substance:	3-Pentenenitrile, purity approximately 100%
Results:	Mortality occurred at ≥ 300 mg/kg within 1 day. Toxic signs observed at lethal doses included unresponsiveness (2250 and 5000 mg/kg), rapid respiration (2250 and 5000 mg/kg), initial weight losses (300, 450, and 670 mg/kg), hyperemic extremities (300, 450, and 670 mg/kg), and polyuria (300, 450, and 670 mg/kg). The non-lethal dose levels of 200, 130, and 90 mg/kg caused initial weight losses. At 200 mg/kg the rat salivated, had hyperemic extremities, polyuria, and was barely responsive on the day of treatment. On the day following treatment, the rat still showed hyperemia and had a rapid respiratory rate; recovery from these signs was evident on the 3rd day after dosing. At 130 mg/kg toxic signs occurred only on the day of treatment and included salivation and polyuria.
Reference:	DuPont Co. (1967). Unpublished Data, Haskell Laboratory Report No. 197-67, "Acute Oral Test" (November 8).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Oral Toxicity:

Data from this additional source were not summarized because the test substance was a mixture or otherwise inappropriate.

DuPont Co. (1982). Unpublished Data, Haskell Laboratory Report No. 656-82, "Oral LD_{50} Test in Rats" (October 19) (also cited in TSCA fiche OTS0555851).

Data from this additional source were not summarized because the study design was not adequate.

Tanii, H. et al. (1989). Neurotoxicology, 10:157-166.

Type:	**Inhalation LC_{50}**
Species/Strain:	Male rats/ChR-CD
Exposure Time:	4 hours
Value:	420 ppm (95% confidence interval, 362-478 ppm)
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Male rats (6/exposure level), weighing 250-289 g, were exposed to nominal concentrations of 338, 359, 447, 538, or 692 ppm 3-pentenenitrile in a 16 L bell jar for 4 hours. The test substance was metered at a uniform rate into a heated stainless steel T-tube by a syringe drive and vaporized under prepurified nitrogen. The test substance vapors were mixed with oxygen and carried into the bell jar. Houseline air was used as diluent to give the desired atmospheric concentration. Gas samples were taken periodically from the chamber exit and analyzed by gas chromatography. Gross and histopathologic examinations were performed on 2 rats each at 1, 2, 7, and 14 days post-exposure. Tissues examined included lungs, liver, spleen, kidney, testes, and thymus. The other survivors were sacrificed 14 days post-exposure.
GLP:	No
Test Substance:	3-Pentenenitrile, purity approximately 100%
Results:	Mortality was 0/6, 2/6, 1/6, 3/6, and 5/6 at 338, 359, 447, 538, and 692 ppm, respectively. Death occurred from 2.5 hours to overnight. Clinical signs observed at lethal concentrations during exposure included irregular respiration, hyperemia, red discharge around the eyes, tremors, salivation, and pale ears. Clinical signs observed at non-lethal concentrations during exposure included irregular respiration, incoordination, red discharge from the nose, and hindleg tremors. Clinical signs observed post-exposure at lethal concentrations included hypersensitivity and initial weight loss followed by normal weight gain. Clinical signs observed post-exposure at non-lethal concentrations included incontinence and initial weight loss followed by normal weight gain. Gross and histopathologic examination revealed no anatomical evidence of primary injury.

Reference:	DuPont Co. (1970). Unpublished Data, Haskell Laboratory Report No. 301-70, "Acute Inhalation Toxicity" (July 15) (also cited in TSCA fiche OTS0555686).
	DuPont Co. (1968). Unpublished Study Data (January 16).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Inhalation Toxicity: None Found.

Type: **Dermal Toxicity:** No Data.

Additional Reference for Acute Dermal Toxicity:

Data from this additional source were not summarized because the test substance was a mixture or otherwise inappropriate.

DuPont Co. (1983). Unpublished Data, Haskell Laboratory Report No. 67-83, "Acute Skin Absorption LD_{50} Test on Rabbits" (March 10) (also cited in TSCA Fiche OTS0570947).

Type:	**Dermal Irritation**
Species/Strain:	Male guinea pigs/Albino
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.
	In a test for primary irritation, applications of 1 drop of undiluted 3-pentenenitrile or of a solution in 1:1 acetone-dioxane containing 13% guinea pig fat (f.a.d.) were applied to the intact shaved skin of male guinea pigs. Reactions for primary irritation were observed at 1 and 2 days.
GLP:	No
Test Substance:	3-Pentenenitrile, purity approximately 100%
Results:	At the 100% concentration, mild erythema was observed in 1 animal and no reaction was observed in 9 guinea pigs at 1 day. Reaction for primary irritation was not observed at 2 days. At 43%, 10/10 guinea pigs were negative for primary irritation after 1 and 2 days.
Reference:	DuPont Co. (1968). Unpublished Data, Haskell Laboratory Report No. 91-68, "Primary Skin Irritation and Sensitization Tests" (September 5).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Dermal Irritation: None Found.

Type:	**Dermal Sensitization**
Species/Strain:	Male guinea pigs/Albino
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. In a test for sensitization potential, an exposure series was given during a 3-week interval. Five guinea pigs received 9 applications of 100% of the test substance, and 5 others received 4 intradermal injections (each 0.1 mL of 0.85% solution in dimethyl phthalate). A 2-week rest period was followed by a challenge test consisting of applications of 100% test substance and 43% solution in 1:1 acetone-dioxane containing 13% guinea pig fat (f.a.d.) to both intact and abraded skin.
GLP:	No
Test Substance:	3-Pentenenitrile, purity approximately 100%
Results:	3-Pentenenitrile (100%) produced mild erythema in 5 and no erythema in 5 guinea pigs in intact skin after 1 day. At 43%, mild erythema in 1 and no erythema in 9 guinea pigs was observed in intact skin after 1 day. 3-Pentenenitrile (100%) produced mild erythema in 6 and no erythema in 5 guinea pigs in abraded skin after 1 day. At 43%, mild erythema in 6 guinea pigs and no erythema in 4 guinea pigs were observed in abraded skin after 1 day. All reactions were negative (100% and 43%) in intact and abraded skin after 2 days. The total number of guinea pigs sensitized by the test substance was 0/10.
Reference:	DuPont Co. (1968). Unpublished Data, Haskell Laboratory Report No. 91-68, "Primary Skin Irritation and Sensitization Tests" (September 5).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Dermal Sensitization: None Found.

Type:	**Eye Irritation**
Species/Strain:	Rabbits/Albino
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Undiluted 3-pentenenitrile (0.1 mL) was instilled into the right conjunctival sac of each of two rabbit eyes. Twenty seconds after contact, 1 exposed eye was washed

	with tap water for 1 minute. The exposed eye of the other rabbit was not washed. Observations were made with a hand slit lamp at 1 and 4 hours and at 1, 2, 3, and 7 days. A biomicroscope was used at examinations at and after 4 hours, and fluorescein stain was used after the day of treatment.
GLP:	No
Test Substance:	3-Pentenenitrile, purity approximately 100%
Results:	Temporary mild corneal injury and conjunctival irritation without significant iritic change was observed. Differences between washed and unwashed eyes were considered within normal variation, and both eyes were normal within 7 days.
Reference:	DuPont Co. (1968). Unpublished Data, Haskell Laboratory Report No. 91-68, "Eye Irritation Test" (September 5).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Eye Irritation: None Found.

5.2 Repeated Dose Toxicity

Type:	**2-Week Inhalation**
Species/Strain:	Rats/ChR-CD
Sex/Number:	Male/6 per exposure level
Exposure Period:	2 weeks (total of 10 exposures)
Frequency of Treatment:	4 hours
Exposure Levels:	0, 55 ppm
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Male rats, weighing 250-289 g, were exposed to 3-pentenenitrile in a 16 L bell jar for 4 hours/day for 2 weeks. The test substance was metered at a uniform rate into a heated stainless steel T-tube by a syringe drive and vaporized under prepurified nitrogen. The test substance vapors were mixed with oxygen and carried into the bell jar. Houseline air was used as diluent to give the desired atmospheric concentration. Gas samples were taken periodically from the chamber exit and analyzed by a gas chromatography. Six control rats were exposed to oxygen and nitrogen for the same amount of time. Three control and 3 test rats were sacrificed after the 10^{th} exposure for gross and histopathologic examination. The remaining animals were sacrificed for gross and histopathologic examination

	following a 14-day recovery period. Tissues examined included lungs, liver, spleen, kidney, testes, and thymus.
GLP:	No
Test Substance:	3-Pentenenitrile, purity approximately 100%
Results:	No deaths occurred during this study. Clinical signs observed during exposure included mild hyperemia and red discharge around the eyes. Normal weight gain was observed post-exposure. Gross and histopathologic examination revealed no evidence of primary injury by the test substance.
Reference:	DuPont Co. (1970). Unpublished Data, Haskell Laboratory Report No. 301-70, "Subacute Inhalation Toxicity" (July 15) (also cited in TSCA fiche OTS0555686).
Reliability:	Medium because a suboptimal study design was used.

Additional References for Repeated Dose Toxicity:

Data from these additional sources were not summarized because the study design was not adequate.

DuPont Co. (1995). Unpublished Data, Haskell Laboratory Report No. 655-95, "Range-Finding Neurotoxicity Study in Rats" (November 9) (also cited in TSCA fiche OTS0557945).

Gagnaire, F. et al. (1998). J. Appl. Toxicol., 18(1):25-31.

5.3 Developmental Toxicity: No Data.

5.4 Reproductive Toxicity: No Data.

5.5 Genetic Toxicity

Type:	***In vitro* Bacterial Reverse Mutation Test**
Tester Strain:	*Salmonella typhimurium* strains TA97, TA98, TA100, TA104, TA1535, and TA1537
Exogenous Metabolic Activation:	With and without 10 and 30% Aroclor®-induced rat and hamster liver S-9
Exposure Concentrations:	Initial Trial: 0, 33, 100, 333, 1000, 3333, 5000, and 6667 µg/plate Subsequent Trials: 0, 33, 100, 333, 667, 750, 1000, 1250, 1500, 2000, 3333, 4000, and 6667 µg/plate
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

The preincubation method originally described by Haworth et al., 1983, was used with some modifications. The test substance, overnight culture of *Salmonella*, and S-9 mix or buffer were incubated at 37°C, without shaking for 20 minutes. Test substances known or suspected to be volatile were incubated in capped tubes. The top agar was added and the contents of the tubes were mixed and poured onto the surface of petri dishes containing medium. Histidine-independent (his+) colonies arising on these plates were counted following 2 days incubation at 37°C. Plates were machine counted (New Brunswick, Artek). At the discretion of the investigator, plates with low numbers of colonies, containing precipitated test substance, or having excessively-reduced contrast because of chemical color, were counted by hand.

The initial test of the test substance was without activation and with 10% S-9. If a positive result was obtained, the positive trial(s) was repeated. If the trials were negative, the test substance was retested without S-9 and with 30% S-9. If all trials were negative, no further testing was performed.

A test substance was designated nonmutagenic only after it had been tested in strains TA97, TA98, TA100, TA1535, and TA1537, without exogenous activation, and with 10% and 30% rat and hamster S-9.

3-Pentenenitrile was run initially in a toxicity assay using TA100 or the system developed by Waleh et al., 1982. Toxic concentrations were defined as those that produced a decrease in the number of his+ colonies, or a clearing in the density of the background lawn, or both.

The test substance was initially tested in the preincubation test at half-log dose intervals up to a dose that elicited toxicity, or to a dose immediately below one that was toxic in the preliminary toxicity procedure. Subsequent trials occasionally used narrower dose increments, and may not have included doses in the toxic range. At least 5 doses of the test substance was tested in triplicate, and repeat experiments were performed at least 1 week following the initial trial.

Concurrent solvent (dimethyl sulfoxide) and positive controls were run with each trial. The positive controls in the absence of exogenous metabolic activation were sodium

azide (TA1535 and TA100), 9-aminoacridine (TA97 and TA1537), and 4-nitro-o-phenylenediamine (TA98). The positive control for exogenous metabolic activation with all strains was 2-aminoanthracene.

The test substance was considered mutagenic or weakly mutagenic if it produced a reproducible, dose-related response over the solvent control, under a single metabolic activation condition, in replicate trials. The test substance was considered questionable if the results of individual trials were not reproducible, if increases in his+ revertants did not meet the criteria for a weakly positive response, or if only single doses produced increases in his+ revertants in repeat trials. The test substance was judged nonmutagenic if it did not meet the criteria for a mutagenic or questionable response.

GLP: Unknown

Test Substance: 3-Pentenenitrile, purity >95%

Results: Equivocal

Remarks: 3-Pentenenitrile was cytotoxic as indicated by a slight clearing of the background lawn and a reduction of revertants starting at concentrations of 3333 µg/plate in the presence and absence of metabolic activation. Weakly mutagenic or equivocal results were produced with and without exogenous activation in *Salmonella typhimurium* strains TA97 and TA100. 3-Pentenenitrile was non-mutagenic with or without exogenous activation in *Salmonella typhimurium* strains TA98, TA1535, and TA1537.

Reference: Zeiger, E. et al. (1992). Environ. Mol. Mutagen., 19(Suppl. 21):2-141.

Haworth, S. et al. (1983) in Environ. Mutagen., 6(Suppl. 1):3-142.

Waleh, N. S. et al. (1982). Mutat. Res., 97:247-256.

Reliability: High because a scientifically defensible or guideline method was used.

Additional Reference for *In vitro* Bacterial Reverse Mutation Studies:

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

DuPont Co. (1978). Unpublished Data, Haskell Laboratory Report No. 753-78, "Mutagenic Activity in the Salmonella/Microsome Assay" (December 19).

Type: ***In vitro* Clastogenicity Studies:** No Data.

Type: ***In vivo* Studies:** No Data.

Appendix D

ROBUST SUMMARY FOR 4-PENTENENITRILE

Existing published and unpublished data were collected and scientifically evaluated to determine the best possible study or studies to be summarized for each required endpoint. In the spirit of this voluntary program, other data of equal or lesser quality are not summarized, but are listed as related references at the end of each appropriate section, with a statement to reflect the reason why these studies were not summarized.

1.0 Substance Information

CAS Number: 592-51-8

Chemical Name: 4-Pentenenitrile

Structural Formula: $CH_2{=}{=}CH{-}CH_2{-}CH_2{-}CN$

Other Names: 3-Butenyl cyanide
4-Cyano-1-butene
4-Pentenonitrile
Allylacetonitrile
1-Cyano-3-butene
Allylmethyl cyanide
4-Pentenoic acid, nitrile

Exposure Limits: No Data.

2.0 Physical/Chemical Properties

2.1 Melting Point

Value:	-47.49
Decomposition:	No Data
Sublimation:	No Data
Pressure:	760 mm Hg
Method:	Modeled. MPBPWIN, v.1.4, module of EPIWIN 3.05 (Syracuse Research Corporation). MPBPWIN estimates melting point by two different methods. The first is an adaptation of the Joback group contribution method for melting point (Joback, 1982; Reid et al., 1987) and the second is a simple Gold and Ogle method suggested by Lyman, 1985.
GLP:	Not Applicable
Reference:	Joback, K. G. (1982). A Unified Approach to Physical Property Estimation Using Multivariate Statistical Techniques. Stevens Institute of Technology, submitted to the Dept. of Chem. Eng. for M.S. Degree at the

Massachusetts Institute of Technology in June 1984 (see also: Reid et al., 1987).

Reid, R. C. et al. (1987). The Properties of Gases and Liquids, 4th edition, Chapter 2, Mc-Graw-Hill, Inc., NY.

Lyman, W. J. (1985). In: Environmental Exposure From Chemicals, Volume I, Chapter 2, Neely, W. B. and G. E. Blau (eds.), CRC Press, Inc., Boca Raton, FL.

Reliability: Estimated value based on accepted model.

SUPPORTING DATA: 1-PENTENENITRILE

Value:	-96°C
Decomposition:	No Data
Sublimation:	No Data
Pressure:	No Data
Method:	No Data
GLP:	No Data
Reference:	Lievens (1924). Bull. Soc. Chim. Belg., 22:127 (Beilstein Database, accessed June 17, 2003).
	Timmermans (1927). Bull. Soc. Chim. Belg., 36:507 (Beilstein Database, accessed June 17, 2003).
	Timmermans and Delcourt (1934). J. Chim. Phys. Phys. Chim. Biol., 31:110 (Beilstein Database, accessed June 17, 2003).
	Joutkovsky (1934). Bull. Soc. Chim. Belg., 43:401 (Beilstein Database, accessed June 17, 2003).
	Witschonke (1954). Anal. Chem., 26:562 (Beilstein Database, accessed June 17, 2003
Reliability:	Not assignable because limited study information was available.

SUPPORTING DATA: 0-OCTADECENENITRILE

Value:	-1°C
Decomposition:	No Data
Sublimation:	No Data
Pressure:	No Data
Method:	No Data
GLP:	Unknown

Reference:	Weast, R.C. (ed.) (1979). Handbook of Chemistry and Physics, 60th ed., p. C-404, CRC Press Inc., Boca Raton, Florida.
Reliability:	Not assignable because limited study information was available.

Additional References for Melting Point: None Found.

2.2 Boiling Point:

Value:	140°C
Decomposition:	No Data
Pressure:	No Data
Method:	No Data
GLP:	Unknown
Reference:	Weast, R. C. (ed.) (1979). Handbook of Chemistry and Physics, 60th ed., p. C-422, CRC Press, Inc., Boca Raton, FL (HSDB/5709).
Reliability:	Not assignable because limited study information was available.

Additional References for Boiling Point: None Found.

2.3 Density

Value:	0.8239
Temperature:	24°C
Method:	No Data
GLP:	Unknown
Results:	No additional data.
Reference:	Weast, R. C. (ed.) (1979). Handbook of Chemistry and Physics, 60th ed., p. C-422, CRC Press, Inc., Boca Raton, FL (HSDB/5709).
Reliability:	No assignable because limited study information was available.

Additional References for Density: None Found.

2.4 Vapor Pressure

Value:	6.36 mm Hg
Temperature:	25°
Decomposition:	Not Applicable
Method:	Estimated using the means of Antoine & Grain methods.
GLP:	Not Applicable

Reference:	SRC MPBPWIN v1.40 in EPIWIN v3.05. Syracuse Research Corporation (MPBPWIN) program estimates the boiling point (at 760 mm Hg), melting point, and vapor pressure of organic compounds. The vapor pressure is estimated using the mean of the Antoine and Grain methods. A description of the methodology is detailed in: *Antoine Method*: Lyman, W. J. et al. (1990). Handbook of Chemical Property Estimation Methods, Chapter 14, American Chemical Society, Washington, DC. *Modified Grain Method:* Lyman, W. J. (1985). In: Environmental Exposure From Chemicals, Volume I, Chapter 2, Neely, W. B. and G. E. Blau (eds.), CRC Press, Inc., Boca Raton, FL.
Reliability:	Estimated value based on accepted model.

Additional References for Vapor Pressure: None Found.

2.5 Partition Coefficient (log Kow)

Value:	1.19
Temperature:	No Data
Method:	Modeled. The KOWWIN computer program, version 1.66 from Syracuse Research Corporation, calculates the Log octanol/water partition coefficient (log Kow) of organic chemicals using an atom/fragment contribution method.
GLP:	Not Applicable
Reference:	The methodology is described in the following journal article: Meylan, W. M. and P. H. Howard (1995). J. Pharm. Sci., 84:83-92.
Reliability:	Estimated value based on accepted model.

Additional References for Partition Coefficient (log Kow): None Found.

2.6 Water Solubility

Value:	6794 mg/L
Temperature:	25°
pH/pKa:	No Data
Method:	Modeled
GLP:	Not Applicable

Reference: WsKow v1.40 in EPIWIN v3.05 (SRC Database).

WsKow estimates the water solubility (WSol) of an organic compound using the compound's log octanol-water partition coefficient (log Kow). The following article describes the estimation methodology:

Meylan, W. M. et al. (1996). Environ. Toxicol. Chem., 15:100-106.

Reliability: Estimated value based on accepted model.

Additional Reference for Water Solubility:

Weast, R. C. (ed.) (1979). Handbook of Chemistry and Physics, 60th ed., p. C-422, CRC Press, Inc., Boca Raton, FL (HSDB/5709).

2.7 Flash Point: No Data.

2.8 Flammability: No Data.

3.0 Environmental Fate

3.1 Photodegradation

Concentration: Not Applicable
Temperature: Not Applicable
Direct Photolysis: Using the absorption spectrum of acetonitrile as an analog example, the nitrile group does not absorp significantly above 200 nm:

absorbance at 200 nm = 0.04
absorbance at 210 nm = 0.03
absorbance at 220 nm = 0.01
absorbance at 254 nm = 0.005.

Harris (1990) also reported that ethylene, an analog for C=C in the unsaturated mononitriles, has no significant absorption above 290 nm. Therefore, the mononitrile category is expected to lack significant absorptivity above 290 nm and will not be subject to direct photolysis.

Indirect Photolysis: In the vapor phase, 4-pentenenitrile is estimated to have an atmospheric half-life of 0.6 days due to hydroxyl radical oxidation and a half-life of 0.96 days due to reactions with ozone. The two reactions result in an estimated atmospheric half-life of 0.37 days for vapor phase material.

Breakdown Products: Not Applicable

Method: The AOP Program, Version 1.90 from Syracuse Research Corporation, estimates the Atmospheric Oxidation Potential. The AOP program estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The methodology used by the Atmospheric Oxidation Program is based upon the structure-activity relationship (SAR) methods developed by Dr. Roger Atkinson and co-workers (Atkinson et al., 1987; 1995; 1996; 1984).

The rate constant for the reaction of 4-pentenenitrile vapor with photochemically generated hydroxyl radicals in the atmosphere is estimated to be $2.6x10^{-11}$ cm^3/molecule-sec at 25°C (SRC AopWin v1.90). This value corresponds to a half-life of 0.6 days, assuming a 24 hour day and an ambient hydroxyl radical concentration of $0.5x10^6$ molecules/cm^3.

GLP: Not Applicable

Reference: Atkinson, R. et al. (1987). Intern. J. Chem. Kinet., 19:799-828.

Atkinson, R. et al. (1995). Atmos. Environ., 29:1685-1695.

Atkinson, R. et al. (1996). Environ. Sci. Technol., 30:329-334.

Atkinson, R. et al. (1984). Chem. Rev., 84:437-470.

Harris, J. C. (1990). Rate of Aqueous Photolysis, Chapter 8, In: Lyman, W. J. et al. (eds.). Handbook of Chemical Property Estimation Methods, American Chemical Society, Washington, DC.

The following journal article describes the AOP Program:

Meylan, W. M. and P. H. Howard (1993). Chemosphere, 26:2293-2299.

Reliability: Estimated value based on accepted model.

Additional References for Photodegradation: None Found.

3.2 Stability in Water

Concentration: Not Applicable

Half-life: The Henry's Law constant for 4-pentenenitrile is estimated to be $2.39x10^{-5}$ atm-m^3/mole (SRC HENRYWIN v3.10 in EPIWIN v3.05) from its estimated vapor pressure of

	6.36 mm Hg (SRC MPBPWIN v1.40 in EPIWIN v3.05, mean of Antoine & Grain methods) and water solubility of 6794 mg/L (WsKow v1.40 in EPIWIN v 3.05). The estimated volatilization half-life from a model river (1 m deep, flowing 1 m/sec, wind velocity of 5 m/sec) is approximately 23 hours. The estimated volatilization half-life from a model lake (1 m deep, flowing 0.05 m/sec, wind velocity of 0.5 m/sec) is approximately 13.6 days (EPIWIN v3.05).
% Hydrolyzed:	Not Applicable
Method:	Modeled. The WVOL program estimates the volatilization half-lives from a model river and lake using the methodology from Lyman et al., 1990 (adsorption to suspended solids and sediments is ignored). The user can input an experimental water solubility, vapor pressure, or Henry's Law constant or EPI will automatically estimate a Henry's Law Constant from SRC's Henry program for this calculation. WsKow estimates the water solubility (WSol) of an organic compound using the compounds log octanol-water partition coefficient (Kow).
GLP:	Not Applicable
Reference:	Lyman, W. J. et al. (1990). The Handbook of Chemical Property Estimation Methods, American Chemical Society, Washington DC. The following journal article describes the estimation methodology: Meylan, W. M. et al. (1996). Environ. Toxicol. Chem., 15:100-106.
Reliability:	Estimated value based on accepted model.

Additional References for Stability in Water: None Found.

3.3 Transport (Fugacity)

Media:	Air, Water, Soil, and Sediments	
Distributions:	Air:	1.5%
	Water:	42.6 %
	Soil:	55.8%
	Sediments:	0.09%
Half-life:	Air:	8.8 hours
	Water:	360 hours
	Soil:	720 hours
	Sediment:	3240 hours

Adsorption Coefficient:	Not Applicable
Desorption:	Not Applicable
Volatility:	Not Applicable
Method:	Calculated according to Mackay, Level III, Syracuse Research Corporation EPIWIN version 3.05. Emissions (1000 kg/hr) to air, water, and soil compartments using standard EPA model defaults with BIOWIN half-life factors of water, 1; soil, 2; and sediments, 9.
	Data Used: Molecular Weight: 81.12 Chemical Name: 4-Pentenenitrile Henry's Law Constant: $2.39x10^{-5}$ atm-m^3/mole (HenryWin Program) Vapor Pressure: 6.36 mm Hg (MPBPWIN v1.40) Log Kow: 1.19 (KowWin Program) Soil Koc: 6.35 (Log Kow estimate)
GLP:	Not Applicable
Reference:	Syracuse Research Corporation EPIWIN v3.05 contains a Level III fugacity model. The methodology and programming approach were developed by Dr. Donald MacKay and coworkers and are detailed in: Mackay, D. (1991). Multimedia Environmental Models: The Fugacity Approach, pp. 67-183, Lewis Publishers, CRC Press. Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1618-1626. Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1627-1637.
Reliability:	Estimated value based on accepted model.

Additional References for Transport (Fugacity): None Found.

3.4 Biodegradation

Value:	Linear Model Prediction: Biodegrades Fast Non-Linear Model Prediction: Biodegrades Fast Ultimate Biodegradation Timeframe: Weeks Primary Biodegradation Timeframe: Days-Weeks MITI Linear Model Prediction: Biodegrades Fast MITI Non-Linear Model Prediction: Biodegrades Fast
Method:	Modeled; BIOWIN v.4.0

GLP: Not Applicable

Reference: The Biodegradation Probability Program (BIOWIN for MS-Windows, v.4) as reviewed by Boethling et al., 1994; Howard et al., 1987; Howard et al., 1992; and Tunkel et al., 2000, used as part of the EPIWIN 3.05 (7/30/02) Suite (Syracuse Research Corporation).

Howard, P. H. et al. (1992). Environ. Toxicol. Chem., 11:593-603.

Howard, P. H. et al. (1987). Environ. Toxicol. Chem., 6:1-10.

Boethling, R. S. et al. (1994). Environ. Sci. Technol., 28:459-65.

Tunkel, J. et al. (2000). Environ. Toxicol. Chem., 19(10):2478–2485.

Reliability: Estimated value based on accepted model.

Additional References for Biodegradation: None Found.

3.5 Bioconcentration

Value: BCF = 1.65. This BCF value suggests that bioconcentration potential in aquatic organisms is low.

Method: The bioconcentration factor is calculated by Syracuse Research Corporation's BCFWIN Computer Program, version 2.14, which utilizes a linear regression based on the Log Kow for the compound.

GLP: Not Applicable

Reference: The estimation methodology used by BCFWIN is described in the following document prepared for the U.S. Environmental Protection Agency (OPPT): "Improved Method for Estimating Bioconcentration Factor (BCF) from Octanol-Water Partition Coefficient," SRC TR-97-006 (2nd Update), July 22, 1997; prepared for Robert S. Boethling, EPA-OPPT, Washington, DC, Contract No. 68-D5-0012; prepared by William M. Meylan, Philip H. Howard, Dallas Aronson, Heather Printup, and Sybil Gouchie, Syracuse Research Corp., Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212.

Reliability: Estimated value based on accepted model.

Additional References for Bioconcentration: None Found.

4.0 Ecotoxicity

4.1 Acute Toxicity to Fish

Type:	**96-hour LC_{50}**
Species:	Fish
Value:	347 mg/L
Method:	Modeled, using log Kow of 1.19.
GLP:	Not Applicable
Test Substance:	4-Pentenenitrile
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Fish: None Found.

4.2 Acute Toxicity to Invertebrates

Type:	**48-hour EC_{50}**
Species:	*Daphnia*
Value:	352 mg/L
Method:	Modeled, using log Kow of 1.19.
GLP:	Not Applicable
Test Substance:	4-Pentenenitrile
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Invertebrates: None Found.

4.3 Acute Toxicity to Aquatic Plants

Type:	**96-hour EC_{50}**
Species:	Green algae
Value:	210 mg/L
Method:	Modeled, using log Kow of 1.19.
GLP:	Not Applicable
Test Substance:	4-Pentenenitrile
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Aquatic Plants: None Found.

5.0 Mammalian Toxicity

5.1 Acute Toxicity

Type:	**Oral ALD**
Species/Strain:	Male rats/ChR-CD
Value:	2250 mg/kg
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. 4-Pentenenitrile, as a solution in peanut oil, was administered in single doses by intragastric intubation to young adult male rats at levels of 200, 300, 450, 670, 1000, 1500, 2250, and 5000 mg/kg. Clinical signs and body weights were recorded. Survivors were sacrificed 14 days later without pathological evaluation.
GLP:	No
Test Substance:	4-Pentenenitrile, purity approximately 100%
Results:	Mortality occurred at ≥ 2250 mg/kg within 1 day. Toxic signs observed at lethal doses included salivation, chewing motions, rapid respiration, and flaccid hindquarters. Rats receiving non-lethal doses of 1500, 1000, and 670 mg/kg had flaccid hindquarters, salivation, and a red nasal discharge. Dose levels of 450, 300, and 200 mg/kg also caused a red nasal discharge on the day of dosing. Weight loss occurred

	at all the non-lethal levels except 200 mg/kg. At 1500 mg/kg, the rat lost weight and was unkempt for 3 days after dosing. At 1000, 670, and 450 mg/kg, the rats lost weight for 2 days, but only an initial weight loss was recorded at 200 mg/kg.
Reference:	DuPont Co. (1967). Unpublished Data, Haskell Laboratory Report No. 198-67, "Acute Oral Test" (November 8) (also cited in TACA fiche OTS0555651).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional Reference for Acute Oral Toxicity:

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Dietz, H. M. et al. (1991). J. Agric. Food Chem., 39(2):311-315.

Type:	**Inhalation LC_{50}**
Species/Strain:	Male rats/ChR-CD
Exposure Time:	4 hours
Value:	2550 ppm (95% confidence limits, 2350-2767 ppm)
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Male rats (6/exposure level), weighing 250-289 g, were exposed to nominal concentrations of 616, 1308, 2265, 4606, 3292 (3292a; 1st exposure at this level), 3292 (3292b; 2nd exposure performed on different animals on subsequent day), 2948, or 2084 ppm 4-pentenenitrile in a 16 L bell jar for 4 hours. The test substance was metered at a uniform rate into a heated stainless steel T-tube by a syringe drive and vaporized under prepurified nitrogen. The test substance vapors were mixed with oxygen and carried into the bell jar. Houseline air was used as diluent to give the desired atmospheric concentration. For analysis, gas samples were taken periodically from the chamber exit and analyzed by a gas chromatographic method. Clinical signs and body weights were recorded during and post-exposure. Gross and histopathologic examinations were performed on 2 rats each at 1, 2, 7, and 14 days post-exposure. Tissues examined included lungs, liver, spleen, kidney, testes, and thymus. The other survivors were sacrificed 14 days post-exposure.
GLP:	No

Test Substance:	4-Pentenenitrile, purity approximately 100%
Results:	The analytical concentrations for the 616, 1308, 2084, 2265, 2948, 3292a, 3292b, 4606 ppm exposure levels were not specified, not specified, 2320, 1990, 2670, 2330, 2925, and 3170 ppm, respectively. Mortality was 0/6, 0/6, 0/6, 1/6, 2/6, 3/6, 5/6, and 6/6 at 616, 1308, 2265, 2084, 2948, 3292a, 3292b, and 4606 ppm, respectively. Death occurred from 2.5 hours of exposure through the night following exposure. At lethal concentrations, irregular respiration, incoordination, lacrimation, salivation, pale ears, tremors, cyanosis, and premortem convulsions were observed during exposure. At non-lethal doses, irregular respiration, incoordination, hindleg tremors, and red discharge from the nose were observed during exposure. Clinical signs observed post-exposure at lethal concentrations were hypersensitivity, and weight loss for 1-2 days followed by normal weight gain. Clinical signs observed post-exposure for non-lethal concentrations included incontinence and initial weight loss followed by normal weight gain. Gross and histopathologic examinations revealed no anatomical evidence of primary injury.
Reference:	DuPont Co. (1970). Unpublished Data, Haskell Laboratory Report No. 301-70, "Acute Inhalation Toxicity" (July 15) (also cited in TSACA fiche OTS0555686). DuPont Co. (1968). Unpublished Study Data (January 16).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Inhalation Toxicity: None Found.

Type: **Dermal Toxicity:** No Data.

Additional Reference for Acute Dermal Toxicity:

Data from this additional source were not summarized because the test substance was a mixture or otherwise inappropriate.

DuPont Co. (1983). Unpublished Data, Haskell Laboratory Report No. 67-83, Acute Skin Absorption LD50 Test on Rabbits" (March 10) (also cited in TSCA Fiche OTS0570947).

Type:	**Dermal Irritation**
Species/Strain:	Male guinea pigs/Albino
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the

	study. In a test for primary irritation, applications of 1 drop of the undiluted sample (100%) or of a solution in 1:1 acetone-dioxane containing 13% guinea pig fat (f.a.d.) (48% based on corrected specific gravity value) were applied to the intact shaved skin of 10 male albino guinea pigs. The reactions were observed after 1 and 2 days.
GLP:	No
Test Substance:	4-Pentenenitrile, purity approximately 100%
Results:	No skin reaction was observed 1 or 2 days after treatment with 100% (observed only 1 day after treatment) or 48% 4-pentenenitrile.
Reference:	DuPont Co. (1968). Unpublished Data, Haskell Laboratory Report No. 92-68, "Primary Skin Irritation and Sensitization Tests" (September 5).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Dermal Irritation: None Found.

Type:	**Dermal Sensitization**
Species/Strain:	Male guinea pigs/Albino
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. In a test for sensitization potential, an exposure series was given during a 3-week interval. Five guinea pigs received 9 applications of 100% 4-pentenenitrile and 5 others received 4 intradermal injections (each 0.1 mL of 95%, based on corrected specific gravity value, solution in dimethyl phthalate). A 2-week rest period was followed by a challenge test consisting of applications of 100% test substance and 48% solution (based on corrected specific gravity value) in 1:1 acetone-dioxane containing 13% guinea pig fat (f.a.d.) to both intact and abraded skin. Sensitization reactions were observed at 1 and 2 days.
GLP:	No
Test Substance:	4-Pentenenitrile, purity approximately 100%
Results:	Sensitization reactions at the challenge phase included 2 and 1 guinea pigs with mild erythema at 100% and 48%, respectively, in intact skin at the 1-day observation. At the 2-day observation for intact skin and at 1 and 2 days for abraded skin no erythema was observed. 4-Pentenenitrile was not a skin sensitizer when tested in albino guinea pigs.

Reference:	DuPont Co. (1968). Unpublished Data, Haskell Laboratory Report No. 92-68, "Primary Skin Irritation and Sensitization Tests" (September 5).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Dermal Sensitization: None Found.

Type:	**Eye Irritation**
Species/Strain:	Rabbits/Albino
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Undiluted 4-pentenenitrile (0.1 mL) was instilled into the right conjunctival sac of each of 2 albino rabbits. Twenty seconds after contact, 1 exposed eye was washed with tap water for 1 minute. The exposed eye of the other rabbit was not washed. Observations were made with a hand slit lamp at 1 and 4 hours, and at 1, 2, 3, and 7 days. Fluorescein stain and a biomicroscope were used at examinations after the day of treatment.
GLP:	No
Test Substance:	4-Pentenenitrile, purity approximately 100%
Results:	4-Pentenenitrile produced mild conjunctivitis of the rabbit eye, but had no significant effect on the iris or cornea.
Reference:	DuPont Co. (1968). Unpublished Data, Haskell Laboratory Report No. 92-68, "Eye Irritation Test" (September 5).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Eye Irritation: None Found.

5.2 Repeated Dose Toxicity

Type:	**2-Week Inhalation Study**
Species/Strain:	Rats/ChR-CD
Sex/Number:	Male/6
Exposure Period:	2 weeks (total of 10 exposures)
Frequency of Treatment:	4 hours per day
Exposure Level:	550 ppm
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

	Six male rats, weighing 250-289 g, were exposed to 4-pentenenitrile in a 16 L bell jar for 4 hours/day for 2 weeks. The test substance was metered at a uniform rate into a heated stainless steel T-tube by a syringe drive and vaporized under prepurified nitrogen. The test substance vapors were mixed with oxygen and carried into the bell jar. Houseline air was used as diluent to give the desired atmospheric concentration. For analysis, gas samples were taken periodically from the chamber exit and analyzed by gas chromatography. Clinical signs were recorded during and post-exposure. Gross and histopathologic examinations were performed, and included lungs, liver, spleen, kidney, testes, and thymus.
GLP:	No
Test Substance:	4-Pentenenitrile, purity approximately 100%
Results:	At 550 ppm, no mortality was observed. Clinical signs during exposure included mild hyperemia and slight irregular respiration. Post-exposure, animals had normal weight gain, and no clinical signs were observed. Gross and histopathologic examination showed no evidence of primary injury by the test substance.
Reference:	DuPont Co. (1970). Unpublished Data, Haskell Laboratory Report No. 301-70, "Subacute Inhalation Toxicity" (July 15) (also cited in TSCA fiche OTS0555686).
Reliability:	Medium because a suboptimal study design was used.

Additional Reference for Repeated Dose Toxicity:

Data from this additional source were not summarized because the study design was not adequate.

Gagnaire, F. et al. (1998). J. Appl. Toxicol., 18(1):25-31.

5.3 Developmental Toxicity: No Data.

5.4 Reproductive Toxicity: No Data.

5.5 Genetic Toxicity

Type: ***In vitro*** **Bacterial Reverse Mutation Studies:** No Data.

Type: ***In vitro*** **Clastogenicity Studies:** No Data.

Type: ***In vivo*** **Studies:** No Data.